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New Developments in Biotechnology

3

Field-Testing Engineered Organisms:

Genetic and Ecological Issues

J952-44

CONGRESS OF THE UNITED STATES
Office of Technology Assessment
Washington, DC 20510-8025

Recommended Citation:

U.S. Congress, Office of Technology Assessment, *New Developments in Biotechnology—Field-Testing Engineered Organisms: Genetic and Ecological Issues*, OTA-BA-350 (Washington, DC: U.S. Government Printing Office, May 1988).

Library of Congress Catalog Card Number 88-600522

For sale by the Superintendent of Documents
U.S. Government Printing Office, Washington, DC 20402-9325
(order form can be found in the back of this report)

Foreword

Since the discovery of recombinant DNA technology in the early 1970s much attention has focused on the potential benefits and risks presented by the new abilities of researchers to manipulate DNA. The importance of ecological issues was heightened in 1982 with the proposal by researchers to field test bacteria engineered to reduce crop losses due to frost damage. Additional pressures have come to bear as a result of developments in the economics of American agriculture and with foreign trade imbalances. In this special report OTA analyzes some of the scientific and public opinion issues surrounding the planned introduction of genetically engineered organisms into the environment.

The assessment of *New Developments in Biotechnology* was requested by the House Committee on Energy and Commerce and the House Committee on Science, Space, and Technology. The first publication in the series was *Ownership of Human Tissues and Cells*, and the second was *Public Perceptions of Biotechnology*. Subsequent studies will examine U.S. investment in biotechnology and issues relevant to the patenting of plants, animals, and micro-organisms. This third report in the series illustrates a range of options for congressional action in three major areas of public policy related to this application of biotechnology:

- the criteria for review of planned introductions for potential risk,
- the administrative mechanisms for applying such review criteria, and
- the research base supporting planned introductions.

In gathering information for this study, OTA staff made site visits to the research facilities or proposed field test sites of seven companies developing engineered organisms for environmental applications or doing similar research. The site visits were made to California, Pennsylvania, Delaware, Missouri, Hawaii, and Wisconsin. Staff also attended and participated in numerous professional meetings devoted to scientific aspects of the issue.

OTA was assisted in preparing this study by a panel of advisors and reviewers selected for their expertise and diverse points of view on the issues covered in the assessment. Advisory panelists and reviewers were drawn from industry, academia, medicine, professional societies, environmental organizations, public interest groups, and Federal agencies. Written comments were received from 140 individuals on successive drafts of the report.

OTA gratefully acknowledges the contribution of each of these individuals. As with all OTA reports, the responsibility for content is OTA's alone.

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NOTE: OTA appreciates and is grateful for the valuable assistance and thoughtful critiques provided by the advisory panel members. The panel does not, however, necessarily approve, disapprove, or endorse this report. OTA assumes full responsibility for the report and the accuracy of its contents.

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Chapter 1

Summary, Policy Issues, and Options for Congressional Action

"The greatest service which can be rendered any country is, to add a useful plant to its culture."

Thomas Jefferson
"The Papers of Thomas Jefferson"
(Accession No. 39161),
Library of Congress, 1800

"One must learn by doing the thing, for although you think you know it you have no certainty until you try."

Sophocles
496(?)-406 BCE

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Chapter 1

Summary, Policy Issues, and Options for Congressional Action

The development in the 1970s of techniques for splicing fragments of DNA from different organisms (recombinant DNA technology) opened up a new science of genetic engineering and a new industry, "molecular" biotechnology. The roots of this new industry lie in practices of animal husbandry, agriculture, and fermentation that extend back for thousands of years. To these ancient practices modern biotechnology adds not only recombinant DNA and cell culture, but also a host of applications using living organisms to make commercial products. These techniques promise to reshape many fields; they are now revolutionizing the pharmaceutical industry and medical diagnosis and treatment.

Commercial biotechnology is advancing into areas that depend on the introduction of genetically engineered organisms into the environment. These applications could improve old tools or produce new ones for many fields, including agriculture, forestry, toxic waste cleanup, mining, enhanced oil and mineral recovery, and others. In some cases, such as pest control or toxic waste management, successful development of biotechnological tools could reduce or phase out dependence on older, more hazardous chemical technologies. It is widely expected that the application of such biological approaches to many human activities will prove more benign to the environment than traditional technologies.

Planned introductions of genetically engineered organisms into the environment, often called *deliberate release*, are not, however, without potential risks. Virtually any organism deliberately introduced into a new environment has a small but real chance of surviving and multiplying. In some small subset of such cases, an undesirable consequence might follow. The complexity of even simple ecosystems makes the precise prediction of such events, and of their consequences, difficult.

This element of uncertainty has led some scientists, public officials, and private citizens to voice concern about the safety of planned introductions. Although there is some consensus in the scientific community that the likelihood of unique or serious problems from planned introductions is quite low, this opinion is not held unanimously. Some scientists cite the beneficial introduction of thousands of species of naturally occurring microbes, plants, and animals that have not adversely affected the environment. Other scientists point out that a small fraction of such introductions have become pests, and suggest that genetic modifications that permit engineered organisms to live in habitats new to them may, in some cases, present similar risks. There is also concern among some scientists that the genetic information newly added to existing species may sometimes produce undesirable changes in their ecological relationships with other species, or, in rare cases, be directly transmitted to other species.

The potential benefits of new biotechnologies have been widely reported. Thus, this report focuses primarily on questions raised by the critics: How do environmental risks from planned introductions of genetically engineered organisms compare to those encountered in the past in agriculture and commerce? How accurately can scientists predict the consequences of planned introductions? What genetic and ecological effects are possible or likely? What scientific and social issues need to be considered in developing risk assessment and mangement procedures? Does the introduction of genetically engineered organisms require new regulatory procedures to protect environmental and public health?

It is important to recognize several conditions that limit generally (though not absolutely) the discussions and conclusions contained in this study:

- **Time Scale:** This study focuses on the near, or foreseeable future, generally within about the next 5 years.
- **Subject Matter:** The issues discussed pertain to small-scale field trials more often than to

large-scale, commercial applications. The degree to which these issues are relevant to large-scale, commercial applications should be revealed by the results of small-scale field tests.

- **Definition:** In this study, the term "genetically engineered organism" is used most often to mean an organism to which genetic material has been added or deleted via recombinant DNA techniques. This usage is not, however, absolute, since some regulatory agenices (e.g., EPA) presently define the term more broadly. Some of the genetic or ecological issues discussed in the study might also apply to organisms produced by means not involving recombinant DNA in the strict sense, such as cell fusion.

It is possible that new types of planned introductions not now under development, or the greater scale of some commercial applications, might introduce questions or concerns in addition to those examined in this study.

Foremost among OTA's conclusions is that **there are reasons to continue to be cautious, but there is no cause for alarm**. Significant areas of uncertainty exist, particularly in the realms of microbial ecology and population dynamics. Widespread environmental or ecological problems do not now seem likely, however, though they could emerge in the future. If events develop other than as planned with a particular introduction, it seems more likely that the introduced organisms might become prematurely extinct, and consequently fail to perform as desired. While increased support for relevant research, both fundamental and applied, will reduce uncertainties, **some questions can be answered only with practical experience**.

Even though the range and complexity of applications of new biotechnologies means that the type of general models used for evaluating the risks from chemicals cannot be transferred easily, **adequate review of planned introductions is now possible**. A review process that involves critical study of planned introductions by experts with relevant knowledge and experience offers confidence of being able to anticipate and prevent most potential problems. As the number of such completed reviews increases one may expect some generalizable conclusions about the safety of different types of introductions. This should enable a consequent streamlining of the review process. And although almost any category proposed for exemption from review can be shown wanting through a hypothetical scenario, it is reasonable to expect that, with experience, broader categories will emerge for which less rigorous levels of review could be defended. Categories that could be examined now, at least for abbreviated review, include:

- organisms that could be produced with previously existing methods (e.g., mutagenesis and selection) which, if they were, would not be regulated under existing law;
- an organism substantially identical to one that has already been reviewed and approved for field testing;
- organisms not containing any genetic material from a potential pathogen; or
- organisms whose DNA contains nothing new but marker sequences in non-coding regions.

Regulatory agencies can and should move promptly to establish at least provisional categories for different levels of review. They should act subsequently to modify and streamline these as experience indicates. This will sometimes be a contentious exercise, but the stakes, in terms of economic potential and environmental protection, are sufficient that there can be no substitute for common sense leavened by caution and appropriate flexibility. To guarantee essential public confidence, this also means that such decisions must be accountable, attributable to specific regulatory bodies, and sustainable by defensible and public reasoning.

With adequate review **none of the small-scale field tests proposed or probable within the next several years are likely to result in an environmental problem that would be widespread or difficult to control**. Indeed, greenhouse or microcosm studies are such inadequate predictors of field performance that in many cases **realistic small-scale field tests are likely to be the only way potential risks from commercial scale uses of genetically engineered organisms can be evaluated**. Assuming such small-scale field tests fail to identify areas of significant concern, there would be no scientific reason not to seek further experience with field tests or applications on a larger scale.

It is important to note that modifying organisms for specific human ends is not new; selective breeders of plants and animals have been transferring genes for millennia, often creating forms through centuries of selection that differ from their original stocks more than the forms produced by recombinant DNA methods. One of the distinguishing characteristics of the new technologies is that they allow scientists to do many of the same things as before with previously undreamed of precision and speed. In evaluating the potential risks associated with these new technologies, the appropriate question is not "How can we reduce the potential risks to zero?" but **"What are the relative risks of the new technologies compared with the risks of the technologies with which they will compete?"** Furthermore, **What are the risks posed by over regulating, or failing to develop fully the new technologies? How do we weigh costs and benefits? How much review is enough?** In most cases the new potential risks will be qualitatively similar to the old risks. Sometimes they will be quantitatively less. The potential benefits to be derived are often substantial.

ANTICIPATED APPLICATIONS

Pending and potential environmental applications of genetically engineered organisms span an enormous range—enormous in terms of engineered organisms, the diverse environments into which they will be introduced, and the functions they are intended to perform (see table 1-1 and app. A).

Many pending or imminent introductions involve minor genetic alterations to modify an existing function in an existing organism. Most involve the activity of the product (protein) of a single structural gene. More than a dozen small-scale field tests have already taken place. Applications for others are pending or anticipated in the near future.

Plants

To decrease crop losses due to weeds, genes have been introduced into several plant species to confer resistance or tolerance to certain herbicides, including glyphosate (Calgene, Davis, CA; Monsanto, St. Louis, MO), sulfonylurea (Du Pont, Wilmington, DE), and atrazine (Ciba-Geigy, Greensboro, NC). Depending on the particular herbicide, this practice could increase or decrease pollution hazards by stimulating changes in patterns of herbicide use.

Plants have also been engineered better to resist disease. Tobacco plants (valuable for the ease with which they can be studied) have been transformed to resist crown gall disease (Agracetus, Middleton, WI). Researchers at Monsanto and at Washington University (St. Louis, MO) have also "vaccinated" tobacco and tomato plants against tobacco mosaic virus by inserting a single virus gene into the plant genome. Similar results with the alfalfa mosaic virus suggest that the same technique may protect many plants against virus-caused diseases.

Pest resistance is another promising area. The delta-endotoxin gene of the well known and much used bacterium *Bacillus thuringiensis* (BT), which encodes a toxin effective against certain caterpillars, has been inserted into tobacco and tomato plants.

Investigators are pursuing many other applications, including drought or saline resistant plants. Recombinant DNA and cell fusion techniques are being used to create new crop varieties, improve the nutritional qualities of some species, and increase algal production of substances used in the food industry. Future endeavors might include enhancing the ability of certain algae to sequester heavy metals from seawater, and introducing genes encoding compounds that protect seeds against insects. Such engineered seed protection could improve long-term storage of certain crops.

Animals

Most recombinant DNA work on animals focuses on altering livestock, poultry, or fish to improve reproductive performance, weight gain, disease resistance, or (livestock) coat characteristics. The types of alterations being pursued and the confined agricultural settings of most altered animals both provide continuity between modern and historical biotechnology.

Table 1-1.—Some Representative Pending and Potential Environmental Applications of Genetically Engineered Organisms

MICRO-ORGANISMS

Bacteria as pesticides. "Ice-minus" bacteria to reduce frost damage to agricultural crops.

Bacteria carrying *Bacillus thuringiensis* toxin to reduce loss of corn crops to black cutworm.

Mycorrhizal fungi to increase plant growth rates by improving efficiency of root uptake of nutrients.

Plant symbionts. Nitrogen-fixing bacteria to increase nitrogen available to plants, and decrease need for fertilizers.

Toxic waste disposal. Bacteria engineered to enhance their existing abilities to degrade compounds found in sludge in waste treatment plants.

Bacteria engineered to enhance their abilities to degrade compounds in landfills, dumps, runoff deposits, and contaminated soils.

Heavy metal recovery. Engineered enhancements possible to several species of bacteria now used to recover metals from low-grade ores (e.g., copper and cobalt).

Pollution control. Possible increased utility of bacteria in purifying water supplies of phosphorus, ammonia, and other compounds.

Viruses as pesticides. Insect viruses with narrowed host specifity or increased virulence against specific agricultural insect pests, including cabbage looper, pine beauty moth, cutworms, and other pests.

Myxoma virus modified so as to restore its virulence against rabbits (which became resistant during early biocontrol efforts in Australia).

Viruses as vaccines. Vaccines against human diseases including:
—hepatitis A and B
—polio
—herpes simplex (oral and genital)
—malaria
—acquired immunodeficiency syndrome
—rabies
—respiratory syncitial virus

Vaccines against animal diseases including:
—swine pseudorabies
—swine rotavirus
—vesicular stomatitis (cattle)
—foot and mouth disease (cattle)
—bovine rotavirus
—rabies (cattle, other mammals)
—sheep foot rot
—infectious bronchitis virus (chickens)
—avian erythroblastosis
—sindbis virus (sheep, cattle, chickens)

Multivalent vaccines. Vaccines possible for antigenically complex diseases such as:
—malaria
—sleeping sickness
—schistosomiasis

PLANTS

Herbicide resistance or tolerance to:
Glyphosate
Atrazine
Sulfonylurea (chlorosulfuron and sulfometuron)
Imidazolinone
Bromoxynil
Phosphinotricin

Disease resistance to:
Crown gall disease (tobacco)
Tobacco mosaic virus (and related viruses)
Potato leaf roll virus

Pest resistance
BT-toxin-protected crops, including tobacco (principally as research tool) and tomato.
Seeds with enhanced anti-feedant content to reduce losses to insects while in storage.

Enhanced tolerance to environmental factors, including:
Salt
Drought
Temperature
Heavy metals

Nitrogen-fixation enhancements
Nonlegumes enhanced to fix nitrogen, independent of association with symbiotic bacteria.

Engineered marine algae
Algae enhanced to increase production of such compounds as B-carotene and agar, or to enhance ability to sequester heavy metals (e.g., gold and cobalt) from seawater.

Forestry
Trees engineered to be resistant to disease or herbicides, to grow faster, or to be more tolerant to environmental stresses.

ANIMALS

Livestock and poultry
Livestock species engineered to enhance weight gain or growth rates, reproductive performance, disease resistance, or coat characteristics.
Livestock animals engineered to function as producers for pharmaceutical drugs, especially of mammalian compounds that require post-synthesis modifications in the cell.

Fish
Triploid salmon produced by heat shock for use as game fish in lakes and streams.
Fish with enhanced growth rates, cold tolerance, or disease resistance for use in aquaculture.
Triploid grass carp for use as aquatic weed control agents.

SOURCE: Office of Technology Assessment, 1988.

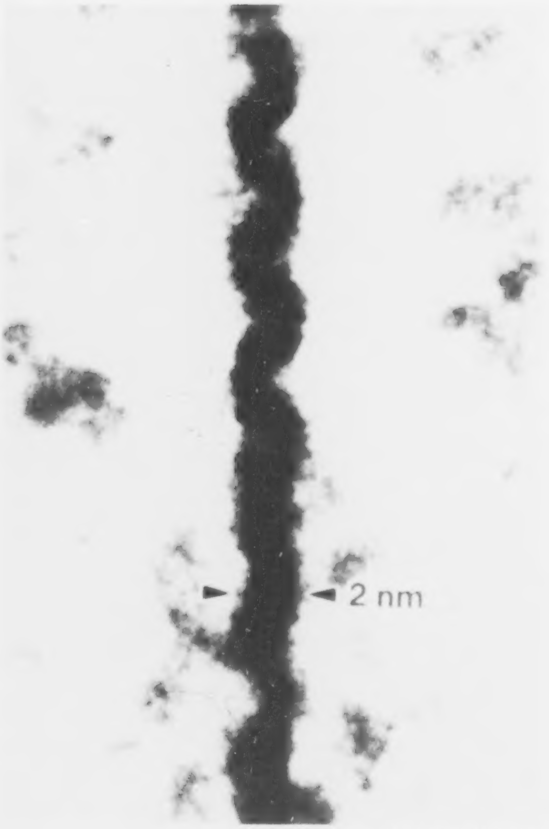


Photo credit: Hideo Yamagishi, Kyoto University

Electron micrograph of DNA double helix, "nm" = nanometer = one-billionth of a meter.

Transferring animal growth hormone genes or exposing fish embryos to abnormally high temperatures to change chromosome number are other successful techniques. Genetic engineering also holds promise for altering insect pests for use in integrated pest management schemes which rely on biological as well as chemical controls.

Micro-organisms

Bacteria, viruses, and fungi offer the molecular biologist some of the best candidates for genetic engineering. Molecular genetics originated in experiments with certain species of these organisms: Of greatest use in this research is the common inhabitant of the human gut, *Escherichia coli*. Other bacteria of medical importance include *Salmonella*, *Streptococcus*, and *Bacillus*. Yeast, *Saccharomyces cerevisiae*, and other fermenters such as *Lactobacillus* have also been important in the evolution of molecular genetics, as have several bacterial and plant viruses. The genetic structure and functioning of these micro-organisms are among the best understood. Microbes are small and easy to handle in the laboratory, and they reproduce rapidly. Enormous numbers can be examined in a short time.

But the special qualities of micro-organisms also present some unique problems. Because they are microscopic, special techniques are required to monitor their survival or dispersal. Their rapid growth rates under favorable conditions and the ability of some species to exchange genetic material make transfer of altered genetic material among some microbes less predictable than among larger organisms.

The ubiquity of microbes (they are literally everywhere; a gram of soil will commonly contain a billion microbes) and their key roles in the fundamental ecological processes of energy flow and nutrient cycles have led to some concerns about the potential for disruption of these processes if large-scale introductions of genetically engineered microbes take place. Some scientists, however, point to the high degree of functional redundancy among members of microbial communities, which acts as a buffer against far-reaching perturbations. They also cite the lack of negative consequences in considerable experience over the last century, during which large numbers of microbes have been introduced into the environment for agricultural and pest control purposes, and for pollution and waste treatment, and conclude from these observations that problems are not likely.

Many promising environmental applications of engineered micro-organisms are being developed. One bacterial application deletes the gene for a cell membrane protein that acts as a nucleus for frost formation. The resulting so-called ice-minus bacteria, derived from species that normally live on plant surfaces, could help reduce crop losses from frost. In other applications, the BT toxin gene has been inserted directly into some bacteria. In one case researchers hope the transformed bacteria, adapted to live on the surfaces of the roots of corn plants, will help reduce corn crop losses to insect larvae. In another, the transformed bacteria are killed and treated to produce a particle containing BT toxin that can then be applied as a topical insecticide. Other bacteria are being engineered to degrade specific toxic compounds found in industrial waste or sludge. Many bacteria can break down toxic compounds naturally, and there is the potential of enhancing their ability to consume petroleum, solvents benzene derivatives, or halogenated hydrocarbons like polychlorinated biphenyls, polybrominated biphenyls, or dioxin, although these applications are likely to be some years away.

One important application that has received a great deal of research attention is enhancing or transferring the capacity to make atmospheric nitrogen biologically usable, or to "fix" nitrogen. If managed well, this holds significant potential for reducing the amount of nitrogen fertilizer required in agriculture, thus reducing the costs and the problems associated with nitrate contamination of runoff water. BioTechnica International, Inc. (Cambridge, MA) is preparing to field test a variety of a naturally occurring bacterium with enhanced nitrogen-fixing ability. It is likely that the more ambitious goal of transferring nitrogen-fixing capacity directly to plants is many years from realization.

Viruses, especially those that affect insects, have potential as pesticides as well as vaccines for both animals and humans. Recent efforts have been directed toward engineering vaccinia viruses to produce vaccines for hepatitis B, herpes simplex, influenza, and hookworm as well as vaccines against such animal diseases as rabies, vesicular stomatitis (a disease of cattle), and others. These are usually produced by using recombinant DNA methods to separate the virus genes that will evoke immune responses in the infected organisms from the genes encoding the proteins responsible for disease. When the latter are removed, a number of the problems historically associated with attenuated or live virus vaccines in common use today are eliminated. Multivalent vaccines, which could be effective against several diseases at once or against complex diseases, are also being developed.

THE ROLE OF PUBLIC PERCEPTION AND THE REGULATORY REGIME

In many ways, the wide range of organisms and potential uses complicates the regulatory picture for the new industry of modern biotechnology because the **critical issues differ from application to application**. Policymakers need to rely on sound scientific review and weigh carefully any potential risks against anticipated benefits of each new planned introduction. **A flexible review process, founded in critical scientific evaluation and adaptable to the requirements of particular cases, can serve industry and the public interest well without being unduly burdensome**.

The Effects of Public Perception

Public perception of the benefits and risks of biotechnology is as likely to influence future industry developments as is formal risk assessment by scientific groups and public officials. When proposing to field test genetically engineered organisms, scientists—whether in academic institutions or industry—must be prepared to work with local citizens and officials. Recent experience in several communities and an OTA-commissioned survey have shown that public opinion is ambivalent and can be vocal with respect to planned introductions of genetically engineered organisms. In several cases public opinion has thwarted or delayed proposed field tests. In other communities opposition has been minimal, and in some cases vocal elements have been supportive. (Ch. 3 includes descriptions of the role of public perception in about a dozen local communities where field tests have been proposed or carried out.)

As in science and technology generally, public interest in the new biotechnologies is high. Although people are concerned about the morality and safety of genetic engineering, they believe that research should continue. Most say they believe the benefits will ultimately justify the risks. Indeed, the public claims to be willing to accept relatively high probabilities of risk to the environment (provided oversight is sufficient) in exchange for the benefits that might accrue from environmental applications of genetically engineered organisms.

The Existing Regulatory Framework

Shortly after recombinant DNA technology appeared and began to be more widely used during the 1970s, concerns were raised about its safety. In an unprecedented move, scientists developing the new techniques met in 1975 at the Asilomar Conference Center (Pacific Grove, CA), and agreed to control stringently their own research until the safety of the new technology could be assured. In 1976, the National Institutes of Health (NIH) issued the first formal guidelines for recombinant DNA research. As research continued, and as scientists learned more about the safety of genetically engineered organisms, initial fears proved excessive, the guidelines were repeatedly revised, and the controls on recombinant DNA research in the laboratory were relaxed.

Some of the safety concerns that have surfaced over the planned introduction of genetically engineered organisms are about issues quite different from those associated with research confined to a laboratory. Numerous ecological issues not relevant to laboratory work become important when applications move beyond the laboratory and into the environment. Assuming regulation is appropriate (an assumption challenged by some), who should regulate planned introduction experiments? How should regulatory agencies assess potential risks? As more and more products reach field-test stage, the need to answer these questions becomes more pressing.

The White House Office of Science and Technology Policy published the Coordinated Framework for the Regulation of Biotechnology in June 1986. This document identifies the agencies responsible for approving commercial biotechnology products (table 1-2) and their jurisdictions for regulating field tests and planned introductions (table 1-3). It describes the regulatory policies of the Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA), and the Food and Drug Administration (FDA), as well as the research policies of the National Institutes of Health (NIH) the National Science Foundation (NSF), EPA, and USDA. The purpose of the Framework is to enable the agencies to "operate in an integrated and coordinated fashion [to] cover the full range of plants, animals, and micro-organisms derived by the new genetic engineering techniques."

Table 1-2.—Agencies Responsible for Approval of Commercial Biotechnology Products

Biotechnology products	Responsible agencies
Foods/food additives	FDA,* FSIS[a]
Human drugs, medical devices, and biologics	FDA
Animal drugs	FDA
Animal biologics	APHIS
Other contained uses	EPA
Plants and animals	APHIS,[c] FSIS,[a] FDA[b]
Pesticide micro-organisms released in the environment	EPA,[d] APHIS[c]
Other uses (micro-organisms):	
Intergeneric combination	EPA,[d] APHIS[c]
Intrageneric combination:	
Pathogenic source organism	
1. Agricultural use	APHIS
2. Nonagricultural use	EPA,[d] APHIS[c]
Nonpathogenic source organisms	EPA Report
Nonengineered pathogens:	
1. Agricultural use	APHIS
2. Nonagricultural use	EPA,[d] APHIS[c]
Nonengineered nonpathogens	EPA Report

*Designates lead agency where jurisdictions may overlap.

[a] FSIS, Food Safety and Inspection Service, under the Assistant Secretary of Agriculture for Marketing and Inspection Services, is responsible for food use.

[b] FDA is involved when in relation to a food use.

[c] APHIS, Animal and Plant Health Inspection Service, is involved when the micro-organism is plant pest, animal pathogen, or regulated article requiring a permit.

[d] EPA requirement will only apply to environmental release under a "significant new use rule" that EPA intends to propose.

SOURCE: 51 Fed. Reg. 23339.

At present, FDA relies on its existing policies for regulating biotechnology products. EPA regulates biotechnology under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). USDA relies on the Plant Pest Act and related statutes, while the Occupational Safety and Health Administration has regulatory authority over certain aspects of biotechnology that relate to workplace safety.

Table 1-3.—Jurisdiction for Review of Planned Introductions

Proposed research	Responsible agencies
Contained research, no release in environment:	
Federally funded	Funding agency,[a]
Nonfederally funded	NIH or S&E voluntary review, APHIS[c]
Foods/food additives, human drugs, medical devices, biologics, animal drugs:	
Federally funded	FDA,[b] NIH guidelines and review
Nonfederally funded	FDA,[b] NIH voluntary review
Plants, animals and animal biologics:	
Federally funded	Funding agency[a], APHIS[c]
Nonfederally funded	APHIS[c], S&E voluntary review
Pesticide microorganisms:	
Genetically engineered:	
Intergeneric	EPA[d], APHIS[c], S&E voluntary review
Pathogenic intrageneric	EPA[d], APHIS[c], S&E voluntary review
Intrageneric nonpathogen	EPA[d], S&E voluntary review
Nonengineered:	
Nonindigenous pathogens	EPA[d], APHIS
Indigenous pathogens	EPA[d], APHIS
Nonindigenous nonpathogen	EPA[d]
Other uses (micro-organisms) released in the environment:	
Genetically engineered:	
Intergeneric organisms	
Federally funded	Funding agency[a], APHIS[c], EPA[d]
Commercially funded	EPA, APHIS, S&E voluntary review
Intrageneric organisms	
Pathogenic source organisms	
Federally funded	Funding agency,[a] APHIS,[c] EPA[d]
Commercially funded	APHIS[c], EPA[d] (if nonagricultural use)
Intrageneric combination	
Nonpathogenic source organisms	EPA Report
Nonengineered	EPA Report[*], APHIS[c]

[*] Designates lead agency where jurisdictions may overlap.

[a] Review and approval of research protocols conducted by NIH, S&E, or NSF.

[b] APHIS issues permits for the importation and domestic shipment of certain plants and animals, plant pests and animal pathogens, and for the shipment or release in the environment of regulated articles.

[c] EPA jurisdiction for research on a plot greater than 10 acres.

[d] EPA reviews federally funded environmental research only when it is for commercial purposes.

Abbreviations: NIH = National Institutes of Health; S&E = United States Department of Agriculture Science and Education; APHIS = Animal and Plant Health Inspection Service; EPA = Environmental Protection Agency.

SOURCE: 51 Fed. Reg. 23305

Several problems—virtually none of them new or unique to biotechnology—face regulators, particularly in the area of planned introductions of genetically engineered organisms:

- **Scope:** Should regulation be product-based, or is there a foundation for basing it on the processes used in producing the products?
- **Definitions:** Common definitions of critical terms such as *deliberate release* into the environment, and *pathogen*, need to be established.
- **Risk Assessment and Management:** Decision makers must perform a complex balancing act—weighing the known risks and benefits of existing technologies against the potential risks and likely benefits of new technologies.
- **Jurisdiction of Federal Agencies Regulating Biotechnology:** Potential conflicts between overlapping jurisdictions of Federal agencies should be prevented from interfering with appropriate regulation.
- **Role of State and Local Governments:** Local zoning and environmental statutes are important determinants of policy that may affect future tests and applications.
- **Public Perception:** Whatever the scientific and regulatory judgments, public perception

will strongly affect the conduct of field tests and the final outcome of commercial applications of biotechnology.

The Coordinated Framework appears to provide means for dealing with most of these problems and others (discussed in ch. 3). As of May 1988, it had been in use for 23 months, and will soon need to be formally evaluated to determine if there are problems it does not cover adequately.

GENETIC CONSIDERATIONS

The planned introduction of genetically engineered organisms that can survive and multiply raises a variety of genetical questions, many of which are relevant only if there is a reasonable probability that the introduced organisms could have a deleterious impact on the environment or public health. The likelihood of such a consequence depends on the nature of the organism and on the new genetic information it carries. It also depends on whether the new genetic material in the altered organism remains where it was inserted, performing as designed, or is transferred to a new location in a nontarget organism, possibly performing in an unanticipated manner. It may therefore be important to consider, where such occurrence is plausible, the probability that novel genetic material will spread beyond the engineered organisms at the release site. The migration of genetic material from one organism to another by means other than germ cells is called horizontal transfer. In bacteria, horizontal transfer is the transmission of genetic information from one contemporaneous bacterial cell to another by whatever means.

What are the potential outcomes of such transfer? Are they beneficial, harmful, or of no consequence? How can the movement of genetic material be observed? What techniques or constraints can limit the frequency or mitigate any potentially adverse consequences of gene transfer? Special considerations exist for each set of organisms.

What Researchers Know About Gene Transfer

Gene transfer between species takes place via a limited number of means, including:

- **Hybridization:** also known as sexual outcrossing, in plants and animals. In animals this is generally impossible except with closely related species; it is common in many groups of plants.
- **Transformation:** the incorporation by bacteria of DNA fragments from the immediate environment.
- **Plasmids:** circles of DNA that are separate and replicate independently from the chromosome within cells. Some are self-mobilizable, and can transmit themselves between compatible cells. Others require the assistance of mobile plasmids to be transferred.
- **Viruses:** nucleic acid (DNA or RNA) packaged in a protective protein coat (unlike plasmids). To reproduce, a virus must infect a cell and take over the host cell's metabolic machinery. Viruses can transfer genetic material between species via "transduction" (see glossary).
- **Transposons (Transposable Elements) and Insertion Sequences:** DNA sequences carrying one or more genes, and flanked by insertion sequences—short DNA fragments that are able move to different places within the cell on the same or a different DNA molecule.

Some of these mechanisms are shared by several classes of organisms, while others are unique to particular groups. Gene transfer is more likely in some groups of bacteria, and less easy to control or predict than hybridization in plants or animals. Except where indicated, the following discussion of gene transfer is more relevant to bacteria than to higher organisms.

Much of what investigators know about gene transfer comes from laboratory experience. Gene transfer between bacteria, for example, is well studied among laboratory populations. Although the frequency of transfer may be relatively low, rapid reproduction and large populations mean that genes can be transmitted into some bacterial populations quite rapidly, if coupled with the appropriate selection pressures. Among insects, evolutionary evidence indicates transfer can occur via transposable elements and viruses, but how often is not known. Similarly, it seems that some horizontal transmission between disparate mammalian families may have taken place in the past, but little evidence exists for transfer in recent times or at appreciable frequencies. Gene transfer among plants by mechanisms other than hybridization has not been well studied, and if it occurs in nature it seems to be quite rare.

The systems of genetic transfer that have been studied were chosen largely for their amenability to laboratory research. They represent only a small sample of what actually occurs in nature. It is clear that with a few exceptions (i.e., between some closely related animal or insect species, between many higher plants. and between some bacterial species), **significant natural obstacles block most gene flow between species**. It is logical to assume, and data support the conclusion, that natural populations of bacteria (generally less dense than laboratory populations) experience lower rates of gene transfer than do laboratory populations. Questions that remain include:

- How extensively do gene transfer mechanisms observed in the laboratory operate in nature?
- What are the genetic and environmental conditions under which novel information could be incorporated into a foreign genome and subsequently expressed?
- How do populations of organisms limit incursion of new genetic material?

Predicting Potential Effects

Several questions are posed by the introduction of a recombinant organism into the environment: What are the conditions that encourage transfer or maintenance of the inserted genes, and how likely is it that genes could be transferred beyond the target site? If transferred, will the new genetic material be expressed? If transferred and expressed, will there be any environmentally significant consequences, positive or negative? (The last question is considered more fully in the section on *Ecological Considerations*.)

Some observers maintain that if it is known that the gene in question will not move about, the potential consequences of gene transfer should not be a concern. Others argue that if the modified organism or gene will cause no problems if it does spread, then estimating the probability of transfer is unnecessary. Both issues must be addressed for a balanced evaluation of the potential consequences of a proposed introduction experiment. A very low probability of transfer multiplied by a moderate probability of hazard if transfer occurs produces a different situation than if both probabilities are very low. Of course, a significant probability of benefit could also offset all or part of any potential risk.

Other observers argue that it should be assumed that any introduced genetic material will eventually be transmitted to nontarget species, and that any consequences should be anticipated. Predicting the consequences and evaluating the risks of deliberate release requires information about intrinsic and extrinsic factors influencing the magnitude, frequency, and stability of gene transfer. **At a minimum, an analysis of the magnitude of gene transfer in those cases where it might be important must address two questions:**

- **How frequently is the genetic material likely to be transferred** to nontarget organisms, especially in comparison to natural background rates?
- **What is the degree of genetic relationship** between the original organism and the nontarget species?

Intrinsic Factors

Intrinsic factors, which are elements of molecular biology, include characteristics of the host organism such as the gene involved, the vector for transferring the gene, and the engineered system itself. Ideally, the biology and natural history of at least these elements would be well described and understood. **The life cycle, natural history, and genetic repertoire of the organism from which a gene is deleted or into which a gene is inserted should be well understood, and the gene itself and the mechanisms controlling its expression in the new host cell should be examined**. Substantial "natural history" needs to be understood before proceeding with planned introduction experiments. Reviewers should be sensitive to the possible expression of novel properties of the host organism.

Because a vector can shuttle genetic material between organisms, familiarity with its host range and behavior is critical. Some vectors transfer genetic material by themselves, but some require outside help. It is important to establish whether the new gene is mobile or immobile in its new host cell. Last, **the whole construct of the introduced gene, new host, and vector material needs to be considered. The intracellular configuration of the new gene in the host organism—where it finally resides in host DNA—is a prime determinant of its potential for movement to new hosts.**

Extrinsic Factors

Extrinsic factors are imposed by the environment. The environment where the engineered organism will be released should be analyzed along with other environments the organism could encounter. Factors to be considered include:

- **Receptivity of Habitat:** Will the engineered host survive to do its job and reproduce once introduced? Natural variation in crucial environmental factors is great. Soil systems, and other natural habitats, may not be sufficently fertile to support the addition of large numbers of engineered organisms. Engineered bacteria will often be at a selective disadvantage when competing with natural populations.
- **Potential Nontarget Recipients:** The most likely nontarget recipients of engineered genetic material are genetically similar organisms; the probability of transfer declines with decreasing similarity or relatedness. If the engineered gene is already present in the recipient environment, concern about transfer beyond the intended host is reduced.
- **Density:** The higher the densities of engineered organisms and potential nontarget recipients, the more likely gene transfer is, although in the absence of selection pressure it will have no consequence.
- **Selection Pressure:** Environmental conditions will determine whether an engineered gene or organism will persist in a population, be expressed, or increase in frequency after introduction. Strong selection for a particular trait—e.g., the presence of an antibiotic—increases the frequency of a gene or genes coding for that trait. Selection pressures vary with each application and depend on the gene involved, how it is regulated and expressed in the host, and its interaction with the environment. While it can be expected that many introductions will be selected against, or at best be selectively neutral, those introductions favored by selection are likely to be most successful.

Monitoring Gene Transfer

Convenient, economical, and effective methods of tracking engineered organisms or the engineered gene(s) they contain are being developed. These can be divided broadly into selective and biochemical methods.

Selective tracking methods work by marking the host chromosome with antibiotic resistance genes or nutritional markers that confer a competitive advantage under specific conditions. When exposed to selection pressure exerted by the antibiotic, for example, organisms carrying the resistance gene survive and can be easily detected.

Such markers must be carefully screened, however, because those that confer an unintended competitive advantage—or that mutate to confer resistance to a whole family of antibiotics—could lead to problems (see box A). On the other hand, resistance genes are already present in many naturally occurring soil micro-organisms (a valuable source of new antibiotics), and antibiotic resistance markers have long been used in studies of root ecology with no apparent ill effects.

When using nutritional markers the host chromosome can be marked with a metabolic gene (e.g., one coding for the production of an enzyme) not normally found in that organism. Monsanto researchers have produced such a system with their insertion of genes for metabolizing the sugar lactose into the soil bacterium they are studying.

Box A.—The Power of Selection Pressure: Antibiotic Resistance Genes in Bacteria

The bacterium responsible for gonorrhea, *Neisseria gonorrhea*, was once highly vulnerable to penicillin. About 30 years ago, penicillin-resistant strains began appearing. Today, local populations of highly resistant bacteria have become common. **These resistant populations are most often centered in places where low doses of ampicillin (a penicillin derivative) are administered continually and indiscriminately as a prophylactic.** The resistance is carried on a plasmid that has been transferred between bacterial species; the identical plasmid has also been found in the intestinal bacterium *Escherichia coli*.

Responding to the decline in effectiveness of penicillin, many physicians have switched to newer, more effective antibiotics. Spectinomycin is an important one that has been used widely to treat sexually transmitted diseases among U.S. military personnel in the Republic of Korea since 1981. There have been increasingly frequent reports, however, of disease strains resistant to this antibiotic. Tests have revealed that most of these strains are susceptible to penicillin as well as to some other antibiotics.

The key factor in this story is selection pressure. Indiscriminate and widespread use of one antibiotic exerts strong and consistent selection pressure on the target populations (in this case, bacteria), favoring survival of organisms resistant to the antibiotic. Substituting a selection pressure caused by one agent for a similar selection pressure caused by another evokes a similar response to the new agent. In the meantime, selection pressure caused by the first agent (penicillin) having been released, the response (which is energetically expensive for the cell to maintain) is likely to be dismantled by selection pressure against energy consumption unnecessary for survival.

The spread of antibiotic resistance factors is the sort of adaptive response any population will manifest in response to strong selection pressure. In bacteria, the rapid, worldwide spread of the initial penicillin resistance was enhanced by highly active vectors—a conjugative plasmid and a transposon. Such combinations of intense selection pressure and actively mobile vectors should be avoided whenever possible. Responses to such problems should take advantage of existing, natural selection and capitalize on it.

SOURCE: Office of Technology Assessment, 1988.

As with antibiotic resistance gene markers, however, this may not always reveal if or how widely the engineered gene or construct may have traveled to other organisms. The movement of genes does not always correspond completely to the movements of the original host organism. To track the engineered gene itself, a selectable marker gene must remain where it is inserted, close to the gene to be tracked; the two would most likely be transferred together (depending on how closely they were linked), making it possible to locate the engineered gene by selecting for and isolating any cells with the marker gene.

Biochemical methods often rely on gene probes made with recombinant DNA techniques. A gene probe is a segment of DNA whose nucleic acid sequence is complementary to the gene of interest, or to a portion of it. The probe is labeled with radioactivity or marked with a dye that can be easily detected in the laboratory. A gene probe will track a gene even if it is separated from a tightly linked selection marker or in an organism that cannot itself be cultured. But to quantify gene transfer would require general tests of all microbes or DNA in the release environment; processing large numbers of samples would be difficult and expensive.

Inhibiting Gene Transfer

As with detection and tracking, techniques to prevent or reduce horizontal gene transfer are not yet well developed. Researchers can either choose or modify the host and/or the vector so that introduced organisms have a low probability of persisting in the environment, of transferring genetic material, or both. Specific choices and modifications will depend on the characteristics of the organisms involved and the purpose for which they are engineered.

Whereas gene transfer may be a legitimate concern in planned introductions of some bacteria, it is unlikely to be a general concern with plant DNA vectors even when the most active plant vectors are used. Nor is gene transfer a concern in organisms engineered by gene deletion, though other traits may then be important.

Reducing or eliminating the use of mobile plasmids and transposons could also help minimize horizontal transfer. A disarmed transposon with its engineered gene could no longer separate and move independently from the chromosome where it was inserted. This approach of "crippling the vector" has been successfully used in transferring the gene for *Bacillus thuringiensis* toxin into another common bacterium, *Pseudomonas fluorescens*; it has also been used to make an immobile vector for insect genetic engineering.

In another approach, an EPA research group is working to construct a "suicide" bacterium designed to persist in the environment only as long as it is needed. The bacterium contains a plasmid carrying a gene that functions only in the presence of the toxic substance the bacterium is designed to clean up; when the toxic substance is no longer available, the plasmid self-destructs. The technique is intended to destroy the plasmid DNA before it can transfer to another host. However, if the host bacterium were killed before the plasmid were degraded, and the plasmid remained intact with its inserted gene, the plasmid DNA could theoretically reinfect another host. But maintaining this assembly costs the cell energy, and any natural mutation that inactivated the system would be favored by natural selection.

As advances in nucleotide chemistry make other techniques possible, new ways of immobilizing vectors and creating restricted and escape-proof hosts are being explored. Fundamental research might be most productive if directed toward increasing understanding of the ecology of different traits. Meanwhile, the genetic implications of introducing any particular organism into the environment are best considered on an individual basis.

ECOLOGICAL CONSIDERATIONS

The major ecological concern over planned introductions of genetically engineered organisms into the environment stems from the potential for unforeseen or long-term consequences. **Although there are enough uncertainties that introductions should be approached with cau-**

tion, a large body of reassuring data, derived chiefly from agriculture, supports the conclusion that with the appropriate regulatory oversight, the field tests and introductions planned or probable in the near future are not likely to result in serious ecological problems.

The results of most planned introductions are likely to be either beneficial, as planned, or neutral. If only because most planned introductions are likely to be agricultural, any negative consequences would most likely involve an agricultural problem, one that might be controlled or mitigated by crop rotation, introduction of new crop strains, or other agronomic practice. In those rare circumstances of a negative impact on a natural community, the consequence seems most likely to be a transitory disturbance of plant or animal community structure, evidenced by fluctuation in numbers or relative abundance.

The worst possible ecological impact a planned introduction could have would be to disrupt a fundamental ecosystem process, e.g., the cycling of a mineral or nutrient, or the flow of energy in an ecosystem; such disruptions are not, however, among the credible consequences of any introduction that seems likely within the next several years. The high degree of functional redundancy among species (particularly microbes) involved with such processes (e.g., nutrient cycles or energy flow) and the resilience and buffering in natural ecosystems are persuasive arguments against the likelihood of such consequences.

Although predicting ecological consequences of planned introductions is complex, researchers and regulators are addressing the questions raised by such introductions. Five criteria have been laid out for evaluating the likelihood of environmental impact:

1. **Potential for Negative Effects:** If it is known that a recombinant organism will have no neg-

Photo credit: Peter Forde, Advanced Genetic Sciences

Advanced Genetic Sciences Researcher Julianne Lindemann spraying "ice-minus" bacteria on strawberry plants in test plot on April 24, 1987. Protective clothing was required by the California Department of Health Services. Note reporters and onlookers in immediate background where coffee and donuts were consumed without hesitation.

Photo credit: Peter Forde, Advanced Genetic Sciences

Advanced Genetic Sciences Researcher Trevor Suslow spraying "ice-minus" bacteria on strawberry plants in test plot in December 1987. Note minimal protective gear.

ative effects, there is no cause for concern. But predicting ecological effects, their probability, and assessing whether they are negative or positive is not always straightforward.

2. **Survival:** If a genetically engineered organism does not survive, it is unlikely to have any ecological impact. It is also unlikely to fulfill the purpose for which it was engineered (unless brief survival was all that was required).
3. **Reproduction:** Some applications require not only survival of the recombinant organism but its reproduction and maintenance. Increasing numbers could, in some settings, increase the possibility of unforeseen consequences.
4. **Transfer of Genetic Information:** Even if the engineered organism itself dies out, its environmental effects could continue if the crucial genetic material were favored by selection and transferred to and functioned in a native species, as described in the preceding section.
5. **Transportation or Dissemination of the Engineered Organism:** A recombinant organism that moves into nontarget environments in sufficient numbers could interact in unforeseen ways with other populations or members of other communities.

Genetically Engineered Organisms and Exotic Species

Although there is much disagreement, some scientists hold that the historical experience presented by introduced exotic species, though imperfect, offers some useful examples of potential problems to guard against in the planned introduction of genetically engineered organisms.

Every environment contains organisms that have originated and evolved elsewhere and arrived in their new habitats either independently or with human assistance. These are called exotics. Some lessons can certainly be learned from experience with exotics, but important differences exist between them and most genetically engineered organisms.

Most engineered organisms being studied with planned introduction in mind will differ from naturally occurring counterparts in only one or a few genes. Scientists already have a great deal of experience with introductions of new agricultural crops or cultivars that differ from previously existing varieties by a small number of genes. And while one or a few genes can have a major impact on such things as host range or pathogenicity, they more often do not. The USDA's Plant Protection Office has recorded over 500,000 introductions since 1898, mostly from outside the United States, including large numbers of plants, insects, and microbes. Although the proportion that has actually become established is not known, there have been occasional negative consequences, sometimes severe or far-reaching. Few, however, have been lasting, and fundamental ecosystem processes and ecological relationships remain intact. Serious consequences have almost invariably been associated with introduced exotics. Table 1-4 summarizes some of the major differences between planned introductions of genetically engineered organisms and exotics.

Most ecologists agree that successful introductions of exotic species, almost by definition, usually exert some effect, even though only a small fraction of all introductions actually result in the establishment and maintenance of a breeding population. Data for insects suggest that perhaps 41 percent of the species successfully introduced in

Table 1-4.—Comparison of Exotic Species and Genetically Engineered Organisms

	Exotic organism[a]	Engineered organism[b]
No. of genes introduced	4,000 to >20,000	1 to 10
Evolutionary tuning	All genes have evolved to work together in a single package	Organism has several genes it may never have had before. These genes will often impose a cost or burden that will make the organism less able to compete with those not carrying the new genes.
Relationship of organism to receiving environment	Foreign	Familiar, with possible exception of new genes

[a] "Exotic organism" is used here to mean one not previously found in the habitat.

[b] "Engineered Organism" is used here to mean a slightly modified (usually, but not always, by recombinant DNA techniques) form of an organism already present in the habitat.

SOURCE: Office of Technology Assessment, 1988.

the United States between 1640 and 1977 have turned into minor pests, even though they had no known detrimental effects in their original habitats. But not all species behave invasively when introduced into a new habitat, and data on introductions are most likely to overestimate impacts, given that organisms not producing an impact are more likely to be overlooked. Experience with exotics suggests that introductions most likely to result in negative consequences include:

- exotics introduced into an environment they can colonize and where they have no potential natural predators or competitors, e.g., herbivores such as goats or rabbits introduced to an island;
- ecological generalists or species that can survive and flourish on many different foods, e.g., insects that can lay eggs on a variety of host plants or parasites that include a wide spectrum of species in their host range; and
- exotics introduced into disturbed ecosystems where natural relationships and constraints have been disrupted.

Although no planned introductions actually reflect any of these scenarios, close analogs can be found in agricultural systems, which are, in an ecological sense, "disturbed." This may make agricultural systems seem especially susceptible to perturbations from unanticipated consequences of a planned introduction. However, as noted earlier, different strategies for managing agricultural systems provide many flexible control and mitigation techniques. Furthermore, the better analogy with experience from introduced agricultural varieties provides additional reassurance.

Potential Impact on Populations or Communities

Local populations or communities can be affected by the introduction of organisms (engineered or not) that are:

- slightly modified forms of resident types,
- forms existing naturally in the target environment but requiring continual supplements to function,
- forms existing naturally elsewhere that have not previously reached the target environment, or
- genuine novelties.

Most anticipated introductions will be slightly modified forms of resident types. Few are likely to be forms existing previously in the target environment that require supplements, since the need for continual supplementation of existing crops (e.g., with fertilizers) is one of the forces impelling development of engineered varieties free of such requirements. Forms existing elsewhere that are new to the target environment bear the greatest similarity to exotic organisms, and thus carry a higher risk of leading to problems. These also promise great benefits, however, as with the introduction of predators to control introduced insect pests. Few genuine novelties are likely in the foreseeable future.

Plant Communities

At present, most plant genetic engineering focuses on introducing into crop plants genes that confer resistance to herbicides or pests, and these alterations are technically among the easiest to accomplish. The market for herbicides and pesticides is profitable and flexible. Modifying other commercially important traits, such as yield components, overall protein production, taste, nutrition, or photosynthetic rates, lies farther in the future.

A prominent concern is that herbicide-resistance genes may spread into weedy relatives of crop plants, most likely by sexual reproduction. If the genes spread to weeds against which the herbicides are targeted, the herbicides become less effective. Such a development would most likely lead to changes in herbicide use patterns or management practices.

One considerable genetic engineering effort against a specific pest involves the insertion into plants of genes coding for toxins from *Bacillus thuringiensis* (BT). These bacterial compounds are highly effective pesticides against the young larvae of butterflies, moths, and some beetles. Farmers have applied BT to their fields in large quantities for decades. Rohm & Haas (Philadelphia, PA) and Monsanto (St. Louis, MO) have already carried out successful field tests in which engineered tobacco and tomato plants to which

BT toxin genes were added gained protection from predation by caterpillars.

The simple presence of large quantities of BT toxin in the environment is not worrisome because it is not toxic to humans or animals, and it decomposes in a relatively short time. Produced inside crop plant tissues, however, BT toxin is protected from environmental degradation, thus extending its persistence as it provides season-long pest control. However, this might introduce a problem of a different kind: such an approach also lengthens the time that less susceptible individuals (e.g., late larvae and adults) of the target species may be exposed to the toxin, and thus subjected to selective forces that can be expected to lead to evolution of resistance in those populations.

How severe this potential problem might be is unclear. BT accounts for only a minor portion of total pesticide use. While forestry and home garden use has increased in recent years, its market share in agricultural use has declined, losing ground to such promising new compounds as synthetic pyrethroids. But if the evolution of resistance to BT is judged to present more than just a problem for product longevity, evolutionary biology offers several possible solutions.

Because resistance will most quickly evolve in pest populations if the toxin is chronically present, distribution of the toxin could be strictly limited to the times and places it is needed. This could be done by limiting the expression of the BT toxin gene to those plant tissues that pest insects habitually forage on, or by inserting regulatory gene sequences that would induce expression of the BT gene only in response to tissue damage caused by the target pest. With progress in understanding gene regulation in plants, such measures may soon be practical.

Another, immediately practical solution is based on the observation that pathogens and pests adapt more quickly to the defenses of prey species in a genetically homogenous community, such as a cultivated field, than in a genetically diverse one. Increasing the variation in the genes controlling defenses against pests should slow the pests' adaptive response. Genetically pest-resistant crop plants could be mixed, for example, with unprotected plants. A smaller proportion of protected plants will exert lower selection pressures on the insect populations, slowing their evolution of resistance. Yet they would still offer enough protection to preempt the growth of swarms of herbivorous insects that cause the most crop damage. This approach is based on a strong theoretical and experimental foundation, and is well within the capability of existing technologies.

Insect Communities

Because they inflict so much damage on agricultural products, insect communities have become the target of recombinant plants, microorganisms, and insect predators engineered to check them. Two representative examples are the bacterium *Pseudomonas fluorescens*, which has been altered to enable corn to resist the black cutworm, and a class of viruses that parasitizes certain pests.

The black cutworm feeds on the roots of corn plants, causing significant corn crop losses. It is vulnerable to BT toxin. Monsanto scientists have used a special vehicle called a transposable element to insert the gene for BT toxin into *Pseudomonas fluorescens*, which lives among corn roots. The transposable element has been altered to make it unlikely that the inserted gene can move beyond its insertion point or leave its *Pseudomonas* host. Preliminary tests suggest that the bacteria do not move beyond the roots they colonize initially, nor are they likely to persist in a field from season to season.

Baculoviruses, rod-shaped viruses that are specific pathogens to one or a few closely related insect species, are being developed to target insect pests, including the cabbage looper and pine sawfly in the United Kingdom. Initial tests involved inserting a marker DNA sequence into the virus to enable scientists to track it. Researchers hope this work will produce a better understanding—and therefore better control—of the dispersal of such altered viruses, as well as of the genetics of host specificity.

Microbial Communities

Microbes can be and are being altered for many uses. Two of the most useful potential applications involve altering root-inhabiting micro-organisms to increase the amount of nitrogen they fix (discussed later in this chapter), and inoculating plants with altered bacteria to enable them to resist frost damage.

The introduction of ice-minus bacteria has been a source of some controversy among the lay public (see ch. 3). The cell membrane of some bacteria contains a protein encoded by a single gene that acts as an efficient nucleus for the formation of ice crystals on the surfaces of plant leaves or blossoms where the bacteria live. Without such a nucleus, ice crystals do not form until about 9 °F below freezing. Crop losses to frost damage in the United States average about $1.6 billion per year. Scientists reasoned that removing the ice-nucleating gene from the bacteria and using them to colonize crop plants could confer a measure of frost resistance on the host plants and eliminate at least a portion of the annual crop loss.

Small-scale field tests of this ice-minus system present little risk: Ice-minus mutants are present in natural bacterial populations. The different strains produced by geneticists have precise genetic alterations, unlike natural ice-minus bacteria, in which any of thousands of genetic changes can produce the ice-minus trait. Nevertheless, some observers of these field tests have been concerned about a possible worst-case scenario, albeit one that could only apply to large-scale uses of ice-minus bacteria.

Natural precipitation depends on ice nuclei for ice or water droplets to condense around and grow big enough to fall as snow or rain. Terrestrial and marine plant material turn out to be more effective than dust particles as ice nuclei, and bacteria growing on plant material may account for a portion of this difference. If ice-minus bacteria were widely applied in agriculture, some claim, the atmospheric reservoir of ice nuclei might grow smaller, changing local or perhaps even global weather patterns. Some possible support for this argument comes from Africa's Sahel desert, where overgrazing has reduced already sparse vegetation. In this scenario, the reduction in ice nuclei due to overgrazing may have contributed, in turn, to decreasing precipitation, further reducing vegetation.

Several different studies suggest, however, that even under a long chain of worst-case assumptions (many of which contradict known facts) the alteration of climatic patterns through large-scale agricultural applications of ice minus bacteria is not likely. Many of these assumptions, however, could benefit from being tested by further research.

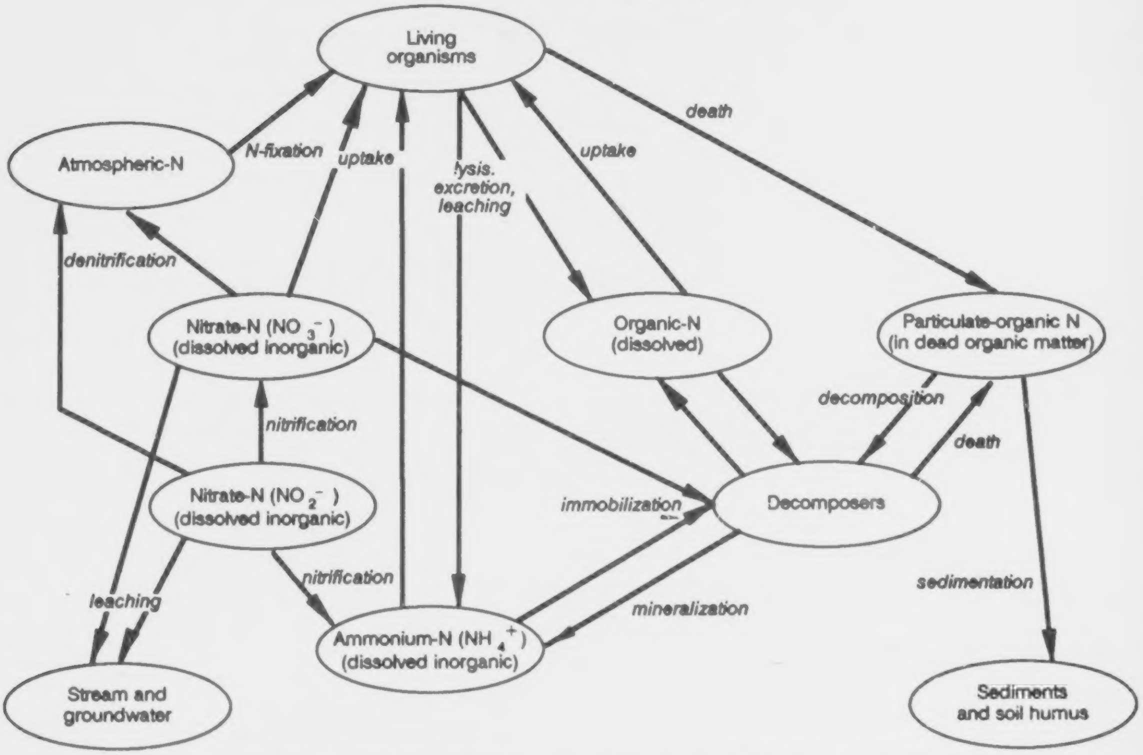
Potential Impact on Ecosystem Processes

Ecosystems are enormously complex and, as a rule, not well understood. Associations of plants, animals, and micro-organisms interact with one another and with their physical environment so as to regulate the flow of energy through the system and the cycling of nutrients within it. The major force driving these processes is capture of the sun's energy by photosynthetic plants and its storage in biologically accessible carbon. Carbon and all other substances vital to living things circulate within ecosystems in biogeochemical cycles. Any major perturbation of these cycles could not only affect living organisms but might disrupt the functioning of ecosystem processes. Much evidence, however, suggests that major perturbation is unlikely.

All organisms require water, carbon, nitrogen, oxygen, phosphorus, and sulfur; carbon and nitrogen are required in the largest amounts, and are usually "rate-limiting." When the biologically available quantity of any of these substances sets an upper limit on the living tissue (biomass) that can be assembled from it and other components, that substance is said to be limiting. When carbon is limiting, as it often is, it puts an absolute upper limit on the biomass a habitat can support. Plants are the primary carbon producers (fixers) in most ecosystems, and decomposers such as insects, nematodes, bacteria, and fungi are the major movers of carbon once it has entered an ecosystem.

The nitrogen cycle (see figure 1-1) is equally important, for plants require nitrogen to grow. Although elemental nitrogen is not limiting—indeed, it makes up nearly 80 percent of the atmosphere by volume—biologically accessible forms of it are. Nitrogen fixation, a complex process that trans-

Figure 1-1.—The Nitrogen Cycle


Living organisms
Atmospheric-N
N-fixation
uptake
lysis. excretion, leaching
uptake
death
denitrification
Nitrate-N (NO_3^-) (dissolved inorganic)
Organic-N (dissolved)
Particulate-organic N (in dead organic matter)
decomposition
nitrification
death
Nitrate-N (NO_2^-) (dissolved inorganic)
immobilization
Decomposers
nitrification
sedimentation
leaching
mineralization
Ammonium-N (NH_4^+) (dissolved inorganic)
Stream and groundwater
Sediments and soil humus

The nitrogen cycle: processes in terrestrial and aquatic systems. The microbially mediated processes include mineralization, nitrification, denitrification, immobilization, and N-fixation.

SOURCE: Office of Technology Assessment, 1988.

forms gaseous nitrogen into biologically accessible forms, is thus crucial to life on earth. The most important nitrogen fixers are rhizobial bacteria, those that live closely associated with the roots of certain plants, particularly legumes.

Given the complexity of ecosystem associations, interactions, and processes, it is not surprising that introducing a genetically engineered organism into the environment has raised concerns. Perturbations of any of the fundamental processes sketched above might have significant effects. Ecosystems, however, by the very complexity that makes them difficult to understand, are buffered and often resilient in the face of perturbations. **While fundamental disruptions of ecosystems should be guarded against, historical experience with both accidental and intentional introductions suggests that such risks are not likely consequences of any planned introductions of genetically engineered organisms being considered now or likely in the near future.**

Nitrogen Fixation

Increasing the availability of nitrogen to economically important plants, thus increasing production per unit cost, would clearly benefit agriculture and forestry. But the biochemical pathways of nitrogen fixation, which appear to be similar in all rhizobial bacteria, are controlled by multigene sequences. Research is under way on these control mechanisms and on transferring nitrogen fixation genes into plants themselves.

The symbiosis between nitrogen-fixing bacteria and their host plants is close. Nearly a century of experience with rhizobial inoculants demonstrates a lack of negative consequences from introductions of such bacteria and makes this a safe system to explore with field tests. Indeed, larger changes to patterns of nitrogen distribution and movement in an ecosystem can generally be achieved by crop rotation than by microbial inoculations.

Larger effects may be possible, however, if the ability to fix nitrogen is transferred to nonleguminous plants. The technical complexity of transferring the large number of genes involved (about 17) and ensuring their effective control are so great that this is not likely within 5 years, and maybe not even in 10.

Microbes and Toxic Waste

Success in enhancing the ability of certain microbes to degrade toxic compounds, including herbicides, pesticides, and industrial wastes, could make a major contribution toward alleviating today's severe toxic waste problem. Naturally occurring microbes with such degradative powers are being used increasingly to help clean up environmental contaminants. On the other hand, introducing a microbe engineered to degrade several classes of materials might conceivably lead to a cascade effect—trigger a chain of consequences arising from chemical similarities between some toxic wastes and natural compounds. It is most likely, however, that bacteria will be engineered to deal with specific compounds, rather than diverse classes. However, if bacteria were engineered to clear up oil spills or grease deposits in sewers, their potential to attack valuable resources might be of concern. But in most microbial environments, carbon is severely limiting, and competition for it is intense. Competition with naturally occurring microbial populations would likely be severe for most engineered microbes. The technical difficulty of producing such constructs makes any associated potential problems unlikely in the near future.

RISK ASSESSMENT

Most researchers and policymakers agree that although there is no general methodology for predicting and evaluating the risks of planned introductions, a flexible, mechanism for review of those that might pose some risk has, for the time being (the next several years), much to recommend it. It offers a high likelihood of anticipating potentially significant problems that might arise and of revealing the kinds of planned introductions that will merit the closest scrutiny as well as those that might require little or none. Review procedures developed for assessing the risks of toxic chemicals have been proposed as models, but it is not clear how applicable these are. With many commercial applications of biotechnology reaching the field-test stage, however, regulators need clear risk assessment and risk management guidelines.

An important element in approaching the current regulatory framework for regulating planned introductions is to distinguish risk assessment from risk management. Risk assessment is the use of scientific data to estimate or predict the effects of exposure to hazardous materials or situations; the process may be qualitative or quantitative. Risk management, on the other hand, is the process of weighing policy alternatives to select the most appropriate regulatory strategy or action. Risk management depends on the scientific findings of risk assessment, but also takes into account technical, social, economic, and political concerns. It is influenced by public opinion and requires value judgments: How acceptable are the potential risks of genetically engineered organisms in the environment relative to their benefits and the costs of controlling them?

Must All Planned Introductions Be Reviewed?

Some scientists and public officials hold that without an adequate, general database for risk assessment of deliberate release experiments, safety can best be ensured by comprehensive scientific review of all proposed releases case-by-case. Others believe that some applications pose such negligible risks that comprehensive review of every proposed field test would be unnecessary as well as burdensome. It is clear that not all planned introductions offer the same potential for undesirable consequences. **It should be possible now, or become possible in the near future, to sort planned introductions into broad categories for which low, medium, or high levels of review are appropriate.** All proposals for field tests will require a certain, minimum level of scrutiny in order to assign them to one of these three levels. What criteria can be used to determine whether an application is inherently safe? Some that are relevant include:

- The engineered organism duplicates the phenotype (appearance and function) and community relationships of naturally occurring organisms or those produced with conventional methods.
- The engineered organism will not survive and reproduce after release into the environment.
- The engineered genetic material will not be transferred to any other organism.
- The engineered genetic, physiological, and ecological functions will have no excessive adverse effects on the environment, or the effects will be unambiguously positive.

As more of the above conditions are met, the level of review might be appropriately reduced. More specific questions also need to be considered. Are there certain categories of organism that are safer to release than others? Are there particular genetic alterations that pose fewer risks than others? Some that have been suggested for abbreviated review include:

- organisms produced by recombinant DNA techniques that are functionally identical to organisms that could be produced by other methods (e.g., mutagenesis and selection, or hybridization) and, if so produced, would not now be subject to regulation;
- organisms containing no genetic material derived from any potential pathogen; or
- any organism identical in function to one that has already been reviewed and approved for field testing.

Genetically engineered domesticated animals and crop plants are widely (though not universally) considered to be relatively safe for release. Given the relative containment of such introductions and the relative ease of controlling them, review of introductions involving agricultural plants and animals might well be kept separate from reviews of introductions involving microbes. Before assuming that a particular animal or plant is safe, however, it is important to ask whether the engineered trait would have any harmful potential in wild populations, and, if so, whether gene flow to related natural populations is possible. The latter will be tightly dependent on whether or not the engineered plant or animal is closely related to and can hybridize with any weedy or otherwise problematic species. In addition, animals or plants that have been altered in a fundamental metabolic function (e.g., a plant made capable of fixing nitrogen) or are partners in mutualisms (interactions that benefit all species involved) also require careful scrutiny.

Engineered organisms that are derivatives of pathogens or pests, differing from the pathogen or pest by only one or two gene changes or organisms engineered to receive from pathogens or pests those genes that are associated with a destructive or disease process, constitute categories likely to require extensive prior review. Most of these are viruses, bacteria, or fungi whose virulence can sometimes shift via simple genetic changes or in which a single gene can determine the difference between a benign and harmful form. Such potential pathogens and pests are unlikely to be exempted from review even though here, too, most genetic changes can be expected not to have effects of this sort. Considerable reassuring experience indicates that many pathogenic organisms can be handled and tested safely (e.g., human and animal vaccines).

In another approach, the molecular details of a genetically engineered organism could be examined to determine whether it could be exempted from review. Such an examination should include but not be limited to these questions:

- Does the introduced organism contain inserted genetic material from a donor of the same genus, or from a different genus? A slightly modified organism might be more likely to persist in the environment into which it was released, leading to greater potential for long-term effects. It is also, however, more likely to have occurred naturally.
- Is the alteration an insertion or a deletion of

genetic material? Deletions generally present less potential than insertions for long-term survival of the organism, or reproduction, and horizontal transfer of an introduced gene.

- Does the alteration involve a regulatory or a structural gene(s)? Regulatory genes control the time, rate, and quantity of production of proteins encoded by structural genes. Changing regulatory sequences could significantly alter an organism's overall structure or functioning, including its ability to survive and reproduce.

In sum, although the characteristics of engineered organisms make certain kinds less likely than others to cause problems, it is not now possible to describe any broad categories that could be completely exempted from review. **Counterexamples can be provided from existing experience to negate almost any proposed category for exemption from review. A review procedure that involves the flexible, adaptable, case-by-case review of proposed planned introductions deemed to involve significant risk is most prudent at present.** Such "case-by-case" language does not imply, however, that each field test should be reviewed *de novo*. Experience with reviewing proposals for planned introductions is rapidly being accumulated. It is reasonable to expect this experience will provide a data base to justify establishing or broadening categories for abbreviated review, eventually to include some exemptions.

Micro-Organisms v. Macro-Organisms

Ecological, genetic, and evolutionary impacts resulting from size differences among organisms must be considered in assessing the risks of their release. Particularly from ecological and evolutionary standpoints, micro-organisms present greater uncertainties than do macro-organisms, though it is not clear this means they present greater risks. Although most macro-organisms are large, and thus relatively easy to track, many insects, weeds, and vertebrates that were introduced have been impossible to exterminate. Most investigators agree that microbes are more difficult to track and control than macro-organisms, though not all agree this means microbes pose greater problems. On the other hand, the life history and population models now available to researchers often fit micro-organisms better than macro-organisms, making them in some ways easier to study.

Charles Robert Darwin, 1809-1882. Discoverer of the Principle of Natural Selection.

Although their large size means macro-organisms can move more biomass or cycle more nutrients through an ecosystem per individual than micro-organisms can, they are not as numerous. The rapid reproductive rates and easy dispersal of small organisms could allow them to proliferate and spread faster through the environment than large ones. And although micro-organisms play key roles in fundamental ecosystem processes, functional redundancy among members of microbial communities seems to provide a greater degree of resilience to environmental perturbations than macro-organisms enjoy.

Evolutionary lability is an important consideration in biological risk assessment: Any assessment of risks, no matter how thorough, would be inadequate should the engineered organisms evolve traits they did not have when released. The potential for a population to evolve depends in part on the numbers of individuals in that population, but most importantly on the selective forces involved. **Therefore all reviews of planned introductions, particularly those involving microbes, should carefully scrutinze the selective forces that will be involved and the likely consequences of selection on the introduced organisms.**

Implications for Research

Some of the controversies surrounding the initial attempts to release genetically engineered organisms into the environment have pointed out gaps in knowledge about ecological systems. Current and proposed small-scale field tests will undoubtedly begin to fill some of these gaps and contribute to the development of better risk assessment protocols, but more than this is needed. Active research cooperation—intellectual, financial, and political—is vital.

Taxpayers are investing much to develop science and technology, but relatively little to develop means for ensuring the safe and wise application of such knowledge. Funding for science and technology, and the resulting research, is very uneven across fields. The National Science Foundation and the National Institutes of Health are major sources for funding in biological research, but the basic knowledge necessary to assess the performance of a technology often remains undeveloped, even as the technology is being refined for use. Research on the ways in which biotechnology may influence natural and managed ecosystems, how to assess its risks and benefits, and how to manage it as a technology, should perhaps be viewed as part of the cost of developing the technology. Research areas that need to be stressed include:

- test systems, such as aquatic and terrestrial laboratory microcosms, where ecological interactions can be analyzed before actual release—although such tests are not sufficient substitutes for field tests, they provide essential information needed in considering the potential consequences of planned introductions;
- the classification and relationships of organisms in natural populations (taxonomy and systematics), especially the genetic relationships of colonizing species or those organisms related to candidates for engineering and planned introductions;
- natural history of organisms planned for genetic alteration and release;
- interactions within natural and managed microbial communities (microbial ecology and population dynamics); and
- more efficient and convenient monitoring and tracking techniques for use in microbial studies;

Interdisciplinary programs involving microbiologists, geneticists, ecologists, evolutionary and molecular biologists, epidemiologists, and risk assessors managed by universities, industry, and government agencies are critical to developing the scientific foundation for setting adequate risk assessment and risk management policies for biotechnology.

POLICY ISSUES AND OPTIONS FOR CONGRESSIONAL ACTION

Three policy issues related to the planned introduction of genetically engineered organisms into the environment were identified during the course of this study. The first involves the development of scientifically founded criteria for the review of planned introductions of engineered organisms. The second concerns actions that Congress might take to shape or direct regulatory policy toward the review and regulation of planned introductions. The third relates to actions Congress might take to affect the development of information and trained personnel that will be needed in the future to ensure that planned introductions continue to be carried out safely.

Following each policy issue several options for congressional action are listed, ranging from taking no specific steps to taking major action. Some of the options involve direct legislative action. Others are oriented to the actions of the executive branch but involve congressional oversight or direction. The order in which options are presented does not imply their priority. Furthermore, the options are not, for the most part, mutually exclusive: adopting one does not necessarily preclude adopting others in the same category or within another category. A careful combination of options might produce the most desirable effects.

ISSUE: What criteria should be used to review applications for permission to field test planned introductions of genetically engineered organisms?

Scientists do not now agree that there is a clear scientific need for a review process by different mechanisms or according to different criteria for engineered organisms intended for environmental introduction than are now being applied to nonengineered organisms.

Option 1: An organism engineered for planned introduction into the environment should not require pre-release review simply because it was produced via recombinant DNA techniques.

With this approach, planned introductions of engineered organisms would not be reviewed according to criteria or mechanisms any different than would be required for the same introduction if it did not involve an engineered organism. A review process organized in accord with this option would have the advantage of focusing exclusively on the product and its characteristics, rather than the process used to produce it. This approach could be most easily adapted to existing regulatory authorities and the mechanisms through which they are administered. One disadvantage is that some potential problems associated with engineered organisms are different than most of the problems existing regulatory authorities handle, e.g., problems stemming from the ability of living organisms to grow, reproduce, or transmit genetic material to nontarget species. It is also possible that some engineered organisms will raise significant, new questions that regulators would overlook, absent special review. However, such problems are not entirely new; some are familiar, already regulated aspects of existing practices, especially in agriculture. Nevertheless, even if there is no clear need for a new regulatory approach, planned introductions of genetically engineered organisms could benefit from some review at least for the foreseeable future, even if only to provide public reassurance that field tests of engineered organisms are not unduly hazardous.

Option 2: All proposals to introduce genetically engineered organisms into the environment should receive the maximum possible prerelease scrutiny.

The advantage of this approach is that it is most likely to ensure that potential hazards associated with field tests of any planned introduction will be discovered and eliminated. The disadvantage is that very few planned introductions, at least for the foreseeable future, seem likely to present significant hazards. Substantial resources could be committed to unnecessary review; the personnel and resources of regulatory agencies would be strained or swamped, and significant impediments would be placed in the path of researchers attempting to develop products.

Option 3: Planned introductions should be reviewed on an adaptable, case-by-case basis, according to scientific criteria that are agreed upon and consistent, and tailored to the specific questions posed by particular applications.

Any specific set of criteria is likely to be somewhat contentious. This will be especially true of any criteria intended to apply to separate proposals that would be reviewed by different agencies. However, the broad outlines of a regulatory approach that should be generalizable are clear: **it should be possible to sort all applications for permission to field test engineered organisms into broad categories for which low, medium, or high levels of prior scrutiny will be appropriate**. Assigning an incoming application to a level of review must, of course, be done on a case-by-case basis. This does not mean that all applications for permission to field test will require the same level of scrutiny: they should not. Nor does it mean that the review of each application should begin *de novo*, without regard to past experience with engineered organisms or relevant knowledge gleaned from the study of nonengineered organisms. Such background information is essential to expeditious review.

As experience accumulates, assuming no untoward developments, since the majority of planned introductions are not expected to generate problems, the presumption of low level review might be extended to a broader range of proposed field tests. Conservative standards that could be useful in sorting proposals into the appropriate review category might include criteria like the following:

- Low Review:
  - —Product is functionally identical to one already reviewed and approved for field testing.
  - —Product is functionally identical to others that can be produced with nonrecombinant DNA techniques.
  - —Product will entail lower levels of risk to the environment or to public health than existing products with which it will compete.
  - —Product differs from naturally occurring organisms only by the addition of noncoding marker DNA sequences to noncoding regions of the DNA of the recipient.
- Medium Review:
  - —Product is different in some ways, but generally similar to previously existing products in general use.
  - —Product entails substantial probability of new genetic material being transmitted to nontarget organisms in application environment or beyond.
  - —Product entails significant probability of altering community into which introduced.
- High Review:
  - —Product involves the transfer into a new host organism of disease genes derived from a pathogenic donor.
  - —Product is a genuine novelty with which there is little or no previous experience that can serve as a guide to risk assessment and management.
  - —Product entails substantial probability of disrupting community into which introduced.

ISSUE: What administrative mechanisms can regulatory agencies use to apply such criteria to the review of applications for permission to field test planned introductions of engineered organisms?

Option 1: Allow regulatory agencies independently to develop and apply criteria for reviewing applications for permission to field test planned introductions of engineered organisms.

This would permit regulatory agencies to develop criteria for sorting and evaluating planned introductions of engineered organism with exclusive attention to applications falling within their separate jurisdictions (e.g., engineered plants by USDA or engineered microbes by EPA). The advantage to this approach is that agencies need not consider issues that would be important only to applications under the jurisdiction of another agency. The drawback to this approach is that different agencies might regulate according to disparate standards or criteria, leading to inconsistent levels of review, regulation, or enforcement.

Option 2: Direct the regulatory agencies to develop, in coordination with one another, but not by any particular process, specific criteria for classifying and reviewing applications for permission to field test planned introductions of engineered organisms.

This was the original intent of the Coordinated Framework established by the Administration on June 26, 1986 (51 Fed. Reg. 23301). In order for this option to function well, however, effective leadership is needed from a coordinating authority. Under the Coordinated Framework, it was intended that this role be fulfilled by the Biotechnology Science Coordinating Committee (BSCC). As it is presently constituted, the BSCC lacks the power to impose its decisions upon the regulatory agencies, or to eliminate disparities in approach by different agencies. Criteria for review that have emerged under this framework to date have not been entirely consistent among agencies. In addition, basic tasks, such as the adoption of commonly agreed upon definitions for "deliberate release," have not yet been accomplished.

Option 3: Provide an interagency group with the power to direct the coordinated development of criteria for classifying and reviewing applications for permission to field test planned introductions of genetically engineered organisms.

This would produce a system similar to that embodied in the Biotechnology Science Coordinating Committee and outlined in the Coordinated Framework, except that the coordinating body would be created by Congress and would have specific powers. Such a body, created by Congress, could be composed of the same or different members as the BSCC, organized within the Office of Science and Technology Policy, or created elsewhere. It would have the authority to direct the preparation of review standards to be used by regulatory agencies according to consistent criteria, and to standardize regulatory approaches as much as possible among different agencies. It could develop such standards independently or in conjunction with the relevant agencies.

ISSUE: Is research supporting the planned introduction of genetically engineered organisms adequate?

Option 1: Take no action.

A significant amount of research is now funded by the Federal Government in areas that contribute to the knowledge base required for sound review and regulation. Principal agencies now sponsoring or conducting such research are NSF, NIH, USDA, and EPA. Such research will likely continue in the absence of additional, targeted appropriations. An example of the type of research likely to be productive is the recent OSTP sponsored initiative, jointly funded by NSF, USDA, and DOE, to fund interdisciplinary, fundamental research in several targeted areas of plant science.

Option 2: Establish an interagency task force to coordinate interdisciplinary research.

Whether or not funding is increased, the different agencies funding relevant research (NSF, USDA, NIH, and DOE) could increase their coordination in the sponsoring of new research intiatives. The recent collaboration between NSF, DOE, and USDA in the establishment of an initiative for research in plant science might be an appropriate model.

Option 3: Increase research funding to selected target areas.

If funding is increased to selected areas, it could most profitably be directed to the divisions of funding agencies sponsoring most of the relevant research. These include, at NSF, the Directorate for Biological, Behavioral, and Social Sciences, and its components, the divisions of molecular biosciences, biotic systems and resources, information science and technology, and others; the National Institute of Environmental Health Sciences and the National Institute of General Medical Sciences at NIH; components of the Division of Science and Education at USDA; and at DOE, the Office of Health and Environmental Research in the Office of Energy Research.

Particular value is likely to be derived from funding earmarked for interdisciplinary studies, or collaborations between scientists in the various disciplines important to understanding and predicting the consequences of environmental perturbations. **Emphasis should be given to studies focusing on the single most important factor affecting the fate and consequences of planned introductions: natural selection**, or the selective interactions between competing organisms, and the selective pressures on organisms due to environmental factors. Other promising areas include basic research in molecular and developmental biology, studies of gene regulation, microbial ecology, community interactions and processes, and evolutionary and ecological relationships.

The disadvantage of such targeting is that it assumes the specific areas where the most important research should be done can be accurately predicted. The results of research are, by nature, unpredictable; this may be especially true of the interdiciplinary research important to planned introductions. Administrative flexibility and adaptability would therefore be important in any such programs, along with the avoidance of undue specificity in the targeting of funds.

Risk assessment and management are vital areas that will increase in importance with the numbers of planned introductions. They lack, however, a strong, vocal constituency to argue for increased funds. The primary agency now funding such studies is EPA, and much of the sponsored research is applied in nature. Both EPA and NSF could be encouraged to enhance their support for basic research relevant to biotechnology research assessment. In the absence of a strong, organized, vocal constituency to help advise on the most effective program, progress might be driven by allocating for risk assessment and management research a fixed proportion of the funding designated for research in other relevant areas.

Public education specific to biotechnology is another important area presently lacking a strong constituency to argue for improvements. They could be achieved through actions taken by the Science Education divisions of both NSF and USDA. Specific measures might include brochures and pamphlets, newsletters, public conferences and debates, yearbooks and annual reports, and extension service activities.

Option 4: Increase personnel education and training.

Because they already have similar programs, the primary agencies to administer any new training programs would logically be NSF, NIH, and USDA. For the near future, the most effective investment would be in programs to provide mid-career training for established investigators. Other valuable programs could include funds for graduate student and postdoctoral training.

There is an urgent need for scientists who are neither molecular biologists nor ecologists, but investigators comfortable with and competent in the techniques and background knowledge of both areas, able to use whichever tools are appropriate to the task. Interdisciplinary training is vitally important to the production of such investigators. Part of the reason there is not more research now being done to develop methods of predictive ecology and risk assessment has to do with historical neglect of these areas by funding agencies, since recognition of their importance has been slow to emerge. But as funding availability has increased in the recent past there has been a relative shortage of investigators applying for or trained to carry out such research.

Chapter 2

Introduction

"The most lasting impact of biotechnology on the food supply may come not from something going wrong, but from all going right. My biggest fear is not that by accident we will set loose some genetically defective Andromeda strain. Given our past record in dealing with agriculture, we're far more likely to accidentally drown ourselves in a sea of excess grain."

Senator Albert Gore, Jr.
National Research Council News Report 37(2):11
February 1987

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Chapter 2

Introduction

This report addresses some of the genetic and ecological questions raised by the planned introduction of genetically engineered organisms. This introductory chapter provides a context for the report's more technical material by recounting the historical background of the issue and reviewing the types of planned introductions either proposed by industry or otherwise likely in the near future.

Of the many consequences of the commercial development of biotechnology, the most far-reaching will likely result from environmental applications of genetically engineered organisms, if only because their sites of application will often be agricultural lands and products. Commonly called "deliberate release," planned introductions of these altered organisms may increase agricultural productivity, aid in the cleanup of toxic wastes, enhance the recovery of minerals from low-grade ores in mining, and provide new applications or enhancements of many existing processes. (The term "planned introduction" will generally be used in this report; other synonyms include: *intentional release*, *deliberate release*, *free release*, and *environmental application*.)

Some observers, however, warn of the potential for damage through unanticipated consequences of a planned introduction. Although the probability of something going awry may be very small in any individual case, the possibility of substantial environmental impact if something should go wrong is not trivial (7,16,19). The world's experience with unanticipated problems related to the petrochemical and nuclear power industries plus recent concerns about disruption of global processes by acid precipitation (22), increased levels of atmospheric carbon dioxide (2,10,12), and other results of chronic environmental alteration suggest such cautionary voices should be listened to. Yet the real similarities between such analogies and biotechnology are scant, and fear should not substitute for reasoned discourse on the potential costs and benefits of a new technology.

Chapter 3 outlines the existing mechanism for regulating planned introductions, and discusses the role of public opinion in shaping regulatory policy. It also describes the experiences of a number of the communities in which early field tests have been proposed, completed, or planned, and recapitulates some relevant results of an OTA-commissioned survey by Louis Harris & Associates (26).

Chapters 4 and 5 summarize and synthesize information on some of the potential consequences of planned introductions of genetically engineered organisms, and the problems that face regulators in estimating the likelihood of such consequences. Chapter 6 discusses risk assessment issues, identifying present capabilities and future needs that must be met to improve risk assessment. A number of technical contract reports were commissioned in support of this study; their titles are given in appendix C.

The potential benefits of biotechnology in general, and of planned introductions of genetically engineered organisms in particular, are widely recognized and described in other studies (4,14, 15). Some sense of this potential may be gleaned from appendix A and table 1-1. This report examines hypothetical negative consequences of such applications. In doing so, OTA seeks to establish whether there are areas of potential concern that might be addressed by legislation that would mandate the assessment and management of planned introductions. Most of the possible negative impacts described are more relevant to perturbations of natural ecosystems than to perturbations of the agricultural systems that will host the majority of imminent environmental applications. As such, negative impacts are not likely to be common consequences of planned introductions in the foreseeable future.

In focusing primarily on questions of potential risk, this report leaves a number of important issues unexamined:

- the economics of research and development (R&D) of planned introductions of genetically engineered organisms;
- the economics of different regulatory ap-

proaches;
- the relationship between R&D and regulation; and
- the potential for social changes and economic rearrangements, as well as a broad range of ethical questions and occupational safety considerations.

Some of these issues are examined in other reports, and it is not clear that biotechnology raises unique questions in these areas.

Developing engineered organisms for specific environmental applications is unlikely to be as expensive as developing a broad-spectrum chemical pesticide, which may cost as much as $50 million (to which regulatory costs contribute significantly). But because the introduction of an engineered organism is likely to be more precise and limited than that of a chemical pesticide—indeed, this is perceived by some as one of the advantages of such engineered organisms—the market over which to amortize the costs of R&D and regulation is small. If the costs of regulating engineered organisms are too high, the development of some applications may be economically unrewarding.

It would be ironic if concerns over the potential impacts of planned introductions, which may be safer than the competing chemical technologies they could displace, lead to such a stringent and expensive regulatory approach that economics forced continued reliance on older, less safe technologies. But if risks sufficient to justify restrictive regulation are identified, it would be logical to extend restraints to existing technologies that entail similar or higher risks.

And while the following chapters suggest that a regulatory system is not strongly grounded in science if it places primary importance on the processes used (e.g., recombinant DNA techniques) rather than the product produced, it cannot be shown that such an approach is entirely without foundation. It is generally agreed that the new techniques will make it possible to do much more quickly many things that were possible before, but only over substantially longer periods. In addition, the new techniques will make it possible to do some things, such as moving genetic material between very different organisms, that would previously not have been contemplated. It will be the challenging task of those who assess and manage risk to determine if these new techniques will eventually bring with them any qualitatively new risks, although it is not now clear that they will.

It is also true that environmental applications of engineered organisms, by increasing yields or productivity in agriculture, may significantly affect economic or social patterns (something that is already taking place independently of biotechnology). Pesticide or herbicide use may be redirected. Growing ranges and seasons of specific crops may shift. Production of specific crops may increase or decrease. The problems and advantages of monocultures and crop diversity may grow or decline. Some of these questions have been studied (25), but none has been approached from the standpoint of genetically engineered organisms in new environmental applications. All these issues could profit from closer examination than is within the scope of this report.

HISTORICAL CONTEXT

Agricultural biotechnology can be traced, some claim, to the earliest domestication of plants and animals in the Middle East, as long as 10,000 years ago (20). Industrial biotechnology is considered by some to follow the prehistoric development of wine making, or the development of brewing in the 11th century. Others see biotechnology dating from the discovery of the structure of deoxyribonucleic acid (DNA) in 1953. Still others date modern biotechnology from the early 1970s, when the tools (restriction enzymes) to move specific pieces of DNA within and between organisms with precision were discovered. These tools greatly increased researchers' ability to intervene in the hereditary processes of plants, animals, and microorganisms. Some individuals see this increase as both a quantitative and qualitative change, affecting not only the amount of intervention possible, but also the kind of effects that can be produced. Regardless of the date chosen as the dawn of biotechnology, the new genetic engineering techniques open possibilities unimagined as recently as two decades ago.

The development of modern biotechnology began, predictably, in research laboratories, where scientists used the techniques to study the structure, function, and organization of genetic material. As technology improved, it became possible to investigate increasingly precise questions about the function of physiological systems and the regulation and interactions of biochemical pathways. Eventually, work with the powerful new tools turned naturally to practical applications, giving rise to the commercialization of biotechnology, a subject examined in an earlier OTA study (23).

The broad applicability of the new techniques is illustrated in the range of industries affected by the emerging biotechnologies. The pharmaceutical industry felt the earliest large effects, with the newly acquired ability to produce significant amounts of such rare or difficult-to-isolate compounds as human growth hormone, insulin, and compounds to dissolve blood clots. Logical extensions of these technologies may result, eventually, in the repair of defective genes in living individuals to cure or ameliorate human genetic diseases (24). The use of monoclonal antibodies (a biotechnology which does not involve recombinant DNA) is expected to revolutionize the diagnosis and treatment of some forms of cancer.

Areas such as specialty chemicals, food additives, commodity chemicals, food processing, waste disposal, mining, and energy production will also experience the effects of biotechnology. Molecular biology and microelectronics may one day meet in a powerful fusion and synthesis, thanks to the new techniques. But agriculture is the next area likely to feel dramatic impacts from biotechnology. Many of these impacts will result from the planned introductions of organisms (mainly plants and microbes) genetically modified to serve precise purposes.

Some observers of these new applications foresee the environmental use of organisms tailored to perform a host of functions. They envision the production of plants that will resist insect pests, disease, and drought; make their own fertilizer; or use nutrients or energy more efficiently. They point to microbes altered to protect crops from frost damage or insect pests; to metabolize toxic wastes contaminating soil or sludge; or to extract rare minerals or compounds more efficiently.

Others point out that we know so little about how our environment actually functions, of how its components interact (or sometimes, even, what those components are), that it is difficult if not impossible to produce a comprehensive, quantitative assessment of the potential risks to the environment from a particular introduction. They dispute the lowest risk estimates of the strongest proponents, and claim that the closest analog to experience with deliberately introducing genetically engineered organisms into the environment is the introduction of exotic plant or animal species into habitats where they were previously unknown. Although more such introductions have been beneficial, or neutral, than harmful, the European starling, gypsy moth, kudzu, Russian thistle (tumbleweed), and cheat grass are well-known examples of negative consequences that can follow when new species are introduced into environments lacking natural checks. If planned introductions could cause similar problems, then potential ecological effects must be scrutinized closely (see ch. 5).

An examination of the pending and potential planned introductions of genetically engineered organisms suggests, however, that **in most cases engineered organisms are not new to the environment in which they will be used.** Almost invariably, either the same organisms or very close relatives already exist in the ecosystem where the proposed application would take place. The major difference between the existing and introduced organisms lies in the addition or alteration of a specific gene or set of genes regulating some aspect of a biochemical pathway.

Instead of likening deliberate release to the introduction of organisms into a new environment, a more reasonable comparison might be the entrance of new genes into existing organisms. There is a long history of selective breeding in plants, animals, and microbes, much of it carried out with no specific knowledge of the mechanisms of heredity or the nature of the hereditary material. Selective breeding has produced organisms that have surely changed environments and ecosystems, but few that are generally agreed to have been deleterious, much less ruinous. Fewer still have been both runaway and drastically negative. These reassuring points were also cited in a recent paper by the National Academy of Sciences (13). But it remains true that most cases of severe environmental trauma seem to have been the logical consequences of intentional activities initially felt to have been unrelated to their eventual effects.

PENDING AND POTENTIAL ENVIRONMENTAL APPLICATIONS OF GENETICALLY ENGINEERED ORGANISMS

How can the potential environmental effects from planned introductions be anticipated? Perhaps by considering the nature and range of a representative sample of anticipated applications. Even the cursory review in this chapter of the pending and potential planned introductions of genetically engineered organisms illustrates that most areas of human life will be touched by these new technologies. (These applications are also referred to throughout the report in the discussions of issues in ecology and genetics that might be of concern.) Microbes, plants, and animals all stand to be affected directly. Most of the pending or potentially near-term applications involve minor alterations to enhance an existing capability or function. Those that do impart genuinely new capabilities are few in number, but even these are fairly simple or straightforward. Most are controlled by only one structural (i.e., protein-producing) gene.

Plants

Applications of genetically altered plants could be numerous and early, and among the least contentious because plant dispersal is comparatively easy to monitor. The genetic changes being made to plants are constrained primarily by technological difficulties peculiar to plants: the relative paucity of methods for inserting DNA, and the peculiar requirements for culturing different plant cell lines. Despite these constraints, numerous companies and researchers are making progress (see app. A).

Genes have been introduced into numerous plant species to confer resistance to herbicides including glyphosate, atrazine, phosphonitricin, the sulfonylureas, and imidazolinones. Some field tests have already been concluded. Other, similar work has transformed different crop plants with genes conferring resistance to one or another antibiotic, a genetic tag that makes the engineered plants easier to monitor.

In addition to herbicide resistance genes, disease resistance genes have been used to transform tobacco plants. The first field tests of plants made resistant to crown gall disease were carried out in 1986.

In a related development, a long known (but incompletely understood) phenomenon has been exploited to provide protection against some plant viral infections. Plants inoculated with mild strains of certain viruses or viroids acquire some protection from more virulent strains that cause severe disease. A collaborative effort between researchers at Washington University in St. Louis, MO, and Monsanto Co. has resulted in this "cross protection" against tobacco mosaic virus being engineered into tobacco plants. In that work, a single viral gene encoding a viral coat protein was inserted into the plant genome. Preliminary indications suggest that the same process might be useful in protecting other plants against a variety of viral diseases.

Many other genetic manipulations to tailor plants for specific environmental applications are being pursued now, or are likely to be pursued in the future. Tolerance to drought, irrigation water salinity, extremes of temperature, and variation in soil conditions all are subject to different degrees of genetic control, making them susceptible to directed manipulations. Because plants engineered for increased tolerance to these fac-

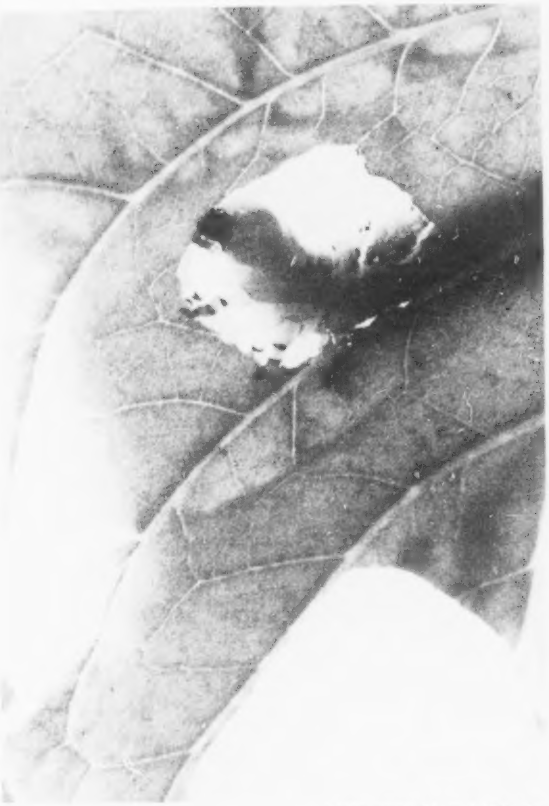

Photo credit: Rohm & Haas Co.

Manduca sexta (tobacco hornworm) larva at work. The moth will consume 95 percent of its entire life cycle's food supply while in the larval stage of development. Moth larvae are the most destructive insects to world agriculture and forestry.

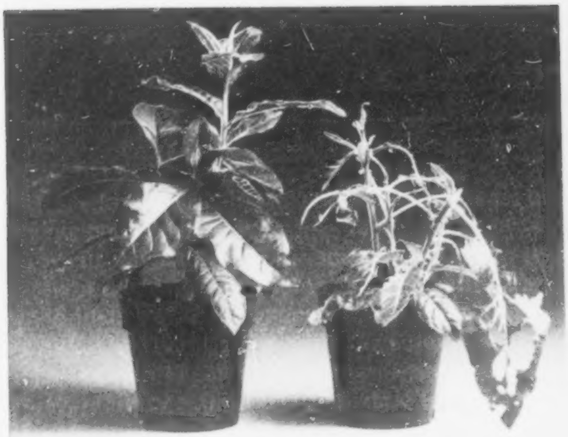

Photo credit: Rohm & Haas Co.

The effects of the BT gene transfer on laboratory tobacco plants can be easily seen in the plant on the left which was infested with 20 tobacco hornworm larvae. Within 40 hours the hornworms were killed by the BT protein in the plant tissue they ingested, leaving the plant virtually undamaged. The other plant, which did not have the gene transfer, shows total destruction by the same number of insects in the same time period.

tors may be grown in habitats new to them, some of the precautions taken to assess the risks of introducing exotic species may apply. Researchers also report substantial progress in the development of recombinant DNA techniques to alter or improve the nutrient qualities of crop plants (9).

Researchers may also explore the possibility of increasing the concentrations of "antifeedants" in seeds. Such compounds (e.g., canavanine), toxic to seed-eating insects, occur naturally in some seeds. Their production is under genetic control, and concentrations vary in seeds from different plant populations and species. This natural variation has already been exploited to reduce losses

Photo credit: Monsanto Co.

Monsanto and Washington University researchers begin field test of tomato plants carrying BT toxin gene in test plot in Jersey County, Illinois, summer of 1987. Examining experimental plants in foreground are Robert Fraley, Director, Plant Science Technology, Monsanto Co., and Roger Beachy, Professor of Biology, Washington University in St. Louis, Missouri.

from insects, particularly in some developing countries. It would be relatively simple to transfer the capacity for producing such compounds to plants that are not naturally able to protect their seeds in this way. Care must be taken, however, that such added compounds do not have negative consequences for the intended consumer, as are known with brown sorghum (6).

In the future, scientists may be able to alter nonleguminous plants to enable them to extract usable nitrogen from the atmosphere (to "fix" nitrogen). However, the technical problems associated with transferring this capability are greater than those posed by almost any of the other applications so far mentioned. While most of those applications involve a single gene, or at most a small number of genes, nitrogen fixation involves as many as 17 different structural genes with associated regulatory elements. To transfer such a gene complex from the parent bacterium into a plant and ensure its proper function in the new genetic background is a challenging task beyond the reach of present techniques. Studies of plant gene regulation are progressing, however. And the first plants with symbiotic bacteria engineered to increase their fixation of nitrogen were ready for testing in 1987, and slated for field tests in 1988.

Other, more tractable plant applications involve the genetic engineering of marine algae to increase their production of food additives (carrageenan, beta-carotene, and agar). Someday algae may even be altered to sequester rare minerals or metals found dissolved in sea water, providing an intriguing fusion of the agriculture and mining industries.

Animals

Because animals are generally larger than microbes, and relatively easy to track, animal applications of genetic engineering have met with less controversy. Most biotechnology work aimed at animals is focused on veterinary products for animals, such as vaccines, or on hormonal supplements like bovine somatotropin. Much of the work that is directed at altering animal genomes per se is geared toward altering farm stock to improve reproductive performance, weight gain, disease resistance, or coat characteristics. Since such organisms (indeed, and many crops, as well) will be restricted to agricultural settings, it will often make more sense to consider them "contained", rather than as introduced into the environment in the way that microbes are. Work is also being done to develop cattle or sheep as "factories" for such substances as human blood factor IX, and other pharmaceuticals.

In work that may be relevant, researchers in Michigan and in Washington are developing strains of fish (salmon) that should live longer and grow larger than average. The procedure involves exposing early fish embryos to abnormally high temperatures. The ensuing shock causes a peculiar chromosomal abnormality (a doubling of one of the chromosomal sets, a condition called triploidy) that disrupts the fish's normal sexual development. The results include infertility as well as longevity and increased size. The altered fish avoid the usual fate of spawning followed by death. Sterile, triploid grass carp are produced in a similar manner, and being used to control some aquatic weeds in Florida. Other researchers (primarily in foreign countries) are working to introduce specific genes into different fish species to increase temperature tolerances or growth rates. Because fish engineered for increased tolerance to such environmental stresses may thrive in habitats new to them, some of the precautions taken to assess the risks of introducing exotic species may apply.

Microbes

Genetically engineered microbes present more uncertainties and generate more concern and opposition than engineered plants or animals. Their small size complicates the task of monitoring and tracking dispersed microbes (5). The genetic promiscuity of some microbes also makes horizontal transfer of genetic material more likely. And microbes are involved in most fundamental ecological processes. But this same involvement, together with the ubiquity of microbes, makes them the choice for many environmental applications of genetically engineered organisms contemplated at present. **An enormous amount of past experience with microbes introduced for biocontrol or agricultural purposes suggests that most in-**

troductions of engineered micro-organisms are likely to be without noticeable ecological consequences. Although most such introductions can be expected to be safe, a few instances of problems with biocontrol microbes used in agriculture suggest the need for some caution (17).

Bacteria are being studied for a host of innovative pesticidal applications. Of great interest is *Bacillus thuringiensis* (BT), a bacterium that produces a protein that is toxic to the larvae of many Lepidoptera (butterflies and moths); different strains produce proteins toxic to some other insect pests, primarily some flies and beetles. A protein known as the delta-endotoxin is produced by a gene that has been inserted into a bacterium (*Pseudomonas fluorescens*) that lives on the roots of corn plants. Scientists hope this will protect corn crops against losses to the black cutworm, which can be substantial in infested fields. Other researchers have inserted the same BT gene directly into plants to exploit its pesticidal properties. In yet another application, the gene has been inserted into a different bacterium that is then killed and preserved in a novel way to increase the toxin's persistence. Under normal circumstances, the ultraviolet-light-sensitive toxin degrades very quickly in the environment; by protecting it from ultraviolet light inside the killed bacterium, the toxin's efficacy as a pesticide is extended.

Viruses also offer potential for exploitation as pesticides. In particular, different baculoviruses (so named for their rod-like shapes) are specific for many insect pests. Genetic engineering to enhance their virulence and limit or alter their host ranges promises to increase their usefulness (21). Early applications of viruses that are pathogenic to insect pests of cabbage plants or pine trees are at or near the field test stage in the United Kingdom, where larvae are serious pests to agriculture and forestry. Research is being carried out on similar systems in several universities and industrial laboratories in the United States.

Other viral applications involve the production of vaccines for both animals and humans. Separate research programs are aimed at tailoring the vaccinia virus to produce vaccines against such diseases. A recombinant vaccine has already been developed for hepatitis B, a major Third World health problem. Other vaccines are being developed for herpes simplex, influenza, hookworm, and AIDS (acquired immunodeficiency syndrome). Animal applications under development include vaccines for vesicular stomatitis (cattle), swine pseudorabies, mammalian rabies, and others. Some of these animal vaccines have undergone field tests, which have generated varying degrees of controversy (see ch. 3).

A number of different groups in the United States and Europe are working on multivalent vaccines designed to protect against several diseases with one vaccination, or against one disease that is antigenically complex (e.g., malaria, sleeping sickness, or schistosomiasis). These programs aim to exploit the large capacity of the vaccinia virus to carry genetic information. This large capacity enables the virus to carry several genes encoding different proteins that will each stimulate an immune response. One of the difficulties in developing vaccines for antigenically complex diseases has been the different antigens the disease agents express at different stages of their life cycles. Traditional vaccines may stimulate an immune response that will protect against infection at one stage in the parasite life cycle, but not another. Vaccinia-based vaccines may overcome this obstacle. Clinical experience with traditional vaccines against many virulent diseases gives excellent reason to suppose that engineered vaccines will be at least as powerful and safe.

Bacteria are also being genetically altered to metabolize specific toxic compounds found in waste or industrial sludge. In recently reported work, scientists have tailored metabolic pathways in bacteria to enhance their ability to metabolize: benzene derivatives (18); the halogenated hydrocarbons (polychlorinated and polybrominated byphenyls (PCBs and PBBs), and dioxin); and oil spills (3,8). The past 5 years have seen much significant progress in this area, using enrichment cultures and naturally occurring bacteria that are then applied to environmental problems. Many naturally occurring bacteria can degrade complex organic compounds, and some are being harnessed to keep closed ecosystems clean, as in the soil bed reactors planned for Biosphere II (1) or in wastewater treatment facilities that must cope with activated sludge.

"Ice-minus" bacteria, the altered bacteria for which the first field test permission was requested, have received the most publicity. These bacteria are expected to reduce frost damage to crops, a problem that costs U.S. agriculture an estimated $1.6 billion annually. Ice-minus bacteria differ from unaltered bacteria in that a single gene has been deleted, one that normally encodes specifications for the construction of a protein normally found in the cell membrane. This protein acts as a potent nucleator for the formation of ice crystals. In the absence of such a nucleator, ice does not commonly form until the temperature drops 5 to 10 °C below freezing. Researchers at the University of California at Berkeley, and at Advanced Genetic Sciences in nearby Oakland, reasoned that the gene for this ice-nucleating protein could be deleted from the bacteria. These altered bacteria—virtually identical to bacteria that can be found in nature—could then replace the normal, ice-nucleation positive (INA +) bacteria living on the leaf surfaces of crop plants, thus providing some protection to frost-sensitive crops. Collaborative work between these groups of researchers has produced early systems designed to protect such crop plants as potatoes and strawberries. Successful field tests of both were conducted in 1987.

An important new marker system called "lac ZY" is being developed to track engineered microbes in the environment. Researchers from Monsanto Co. and Clemson University are developing the system through a collaborative effort (11).

SUMMARY

Planned introductions of genetically engineered organisms span an enormous range in terms of the altered organisms, the diverse environments in which they are to be applied, and the types of functions they are intended to perform. There are difficulties in estimating precisely the potential environmental hazards, and in assessing the risks and benefits for any particular application. But there is also a substantial body of relevant experience that can be used as a guide.

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Chapter 3

The Role of Public Perception and the Regulatory Regime

"In the realm of controlled release, it seems, we need sociology as well as ecology. But the qualifier "good" is essential in each case too."

Bernard Dixon
Bio/Technology 4:481, June 1986

"Public opinion, in today's democracies forms an archipelago, not a continent."

Jean-Francoise Revel
How Democracies Perish, p. 14
Harper & Row, New York, 1983

"The public does not have to understand what is going on in biotechnology. . . . But people need a perception that somebody is in charge and somebody is looking out for their best interests."

Warren Hyer
Bio/Technology 4:497, June 1986

"At this stage of its emergence, the biotechnology industry can learn from the painful experience of the chemical industry: That a public enthralled by technological advances can become quickly hostile if they are not honestly informed about both the benefits and potentially hazardous side effects and then given adequate opportunity to participate in regulatory decision making."

Jay D. Hair
Conservation Exchange 4(4):2, Winter 1986-87

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Chapter 3

The Role of Public Perception and the Regulatory Regime

Field tests involving the deliberate release of genetically engineered organisms into the environment have resulted in increased public interest in and scrutiny of the developing biotechnology industry. The role of government has likewise increased. The United States has developed a Coordinated Framework for the Regulation of Biotechnology (10); the Organization for Economic Cooperation and Development (OECD), representing 24 nations, has developed proposals outlining safety considerations for applications of recombinant DNA organisms in industry, agriculture, and the environment (24); and several European countries have developed laws or regulations governing genetic engineering (7).

Activists opposed to environmental applications of genetically engineered organisms have increased their visibility at the Federal, State, and local levels. Perhaps best known is the Foundation on Economic Trends, led by Jeremy Rifkin, which has filed several biotechnology-related legal challenges. Local groups, some in concert with the Foundation, have become involved in debating the merits of several proposed field tests. Scientists and their constituent organizations have also participated in the public discussion. Public knowledge and opinion about issues concerning science and technology in general and genetic engineering and biotechnology in particular are likely to shape future debate.

This chapter reviews the general public's perceptions as measured by a national survey conducted by Louis Harris and Associates for OTA, the role of public perception in local communities where field tests have been proposed, and the existing governmental framework for the regulation of biotechnology.

ATTITUDES AMONG THE GENERAL PUBLIC

In addition to scientific considerations, public perceptions of the risks and benefits of biotechnology can play an important role in planned introductions of genetically engineered organisms. As one writer has stated, "the public is a mixed bag of people, each of whom interprets the information generated by a biotechnology company based on a set of personal biases" (20). This, combined with the general lack of scientific knowledge with respect to these introductions and the natural sense of unease with which the public views high technology, serves to highlight the need for sound information on public opinion.

Such information can provide a basis for understanding and anticipating public responses to risk factors. It can also identify the quality and sources of information the public draws upon, and provide a basis for improving the communication of risk information among lay people, technical experts, and decision makers (25).

As part of a broader survey, OTA commissioned Louis Harris & Associates to conduct a survey of public opinion on a number of issues related to planned introductions of genetically engineered organisms. The survey consisted of a national sample of 1,273 adults telephoned in November 1986. The variance for this survey is less than 3 percent for the total sample.

The results illustrate the complexities and contradictions characteristic of public attitudes in this area. As the complete data have been published separately (30), only a brief description of the points most germane to deliberate release is presented here.

The American public is interested in biotechnology and genetic engineering. Two-thirds of the public (66 percent) feel they understand the meaning of the term "genetic engineering." In a related question 35 percent say they have heard or read a fair amount about the subject. However, only 19 percent has heard of any potential risks posed by products of genetic engineering. A much higher share (52 percent) believes it to be at least somewhat likely that these products will present some serious danger to humans or the environment. In spite of this, a clear majority (66 percent) thinks genetic engineering will bring changes that will improve their quality of life.

The American public is more positively and consistently inclined toward genetic manipulation of plants, animals, and microbes than toward human cell manipulation. This may in part reflect a sense that "we have no business meddling with nature"—a feeling strongly expressed by 26 percent of the population (table 3-7).

Not only is there some concern about the morality of genetically manipulating organisms, there is also concern about the potential risks that may be posed. Between 52 and 61 percent say they think it is at least somewhat likely that untoward consequences (antibiotic-resistant diseases, human birth defects, herbicide-resistant weeds, or endangered food supply) could follow. But fewer than one in five think any of these developments are very likely (table 3-1).

People seem willing to accept relatively high rates of environmental risk in exchange for the potential benefits that might be derived from deliberate release of genetically engineered organisms. A majority (55 percent) would approve an application that would significantly increase agricultural production even if the risk of losing some local species of plant or fish were as high as 1 in 1,000. With lower levels of risk, the degree of public acceptance increases. But despite a general public willingness to approve the use of genetically engineered organisms in the environment at relatively high levels of risk, a majority says it would not approve an application if the risk were unknown. Indeed, significantly fewer say they would approve if the risk were "unknown but very remote" than would approve if the risk were 1 in 1,000 (45 v. 55 percent) (table 3-2).

If there were no direct risk to humans and only very remote risks to the environment, a majority would approve the planned introduction of genetically engineered organisms to produce disease-resistant crops (73 percent), oil-eating bacteria to clean up spills (73 percent), frost-resistant crops (70 percent), more effective pesticides (56 percent), or larger game fish (53 percent) (table 3-3).

However, this approval is limited. Although a large majority of the public (82 percent) approves of small-scale experimental tests of genetically engineered organisms for environmental applications, 53 percent feel that large-scale experimental tests should not be permitted (table 3-4).

Most Americans also say they would favor or be indifferent to having genetically altered organisms tested in their community, assuming there was no direct risk to humans and a very remote potential risk to the local environment (table 3-4).

Thus, Americans seem to be pragmatic in judging genetic engineering. They are concerned about the morality and the safety of these new developments, but are willing to greet biotechnology with

Table 3-1.—Likelihood of Specific Dangers From Use of Genetically Altered Organisms in the Environment[a]

Q.: From what you have heard or read, how likely do you think it is that the use of genetically engineered organisms in the environment will (READ ITEM)—very likely, somewhat likely, somewhat unlikely, very unlikely?

	Very likely	Somewhat likely	Somewhat unlikely	Very unlikely	Not sure
Create antibiotic-resistant diseases	18%	43%	21%	7%	11%
Produce birth defects in humans	18	39	24	10	9
Create herbicide resistant weeds	15	41	22	11	11
Endanger the food supply	14	38	29	13	7
Mutate into a deadly disease	13	33	30	14	10
Change rainfall patterns	12	30	30	16	12
Increase the rate of plant or animal extinction	11	34	31	15	9

[a] Percentages are presented as weighted sample estimates. The unweighted base from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

Table 3-2.—Acceptable Levels of Risk for an Environmental Application of Genetically Engineered Organisms[a]

Q.: Suppose that a new genetically engineered organism had been developed which would significantly increase farm production with no direct risk to humans. Would you approve the environmental use of that organism if the risk of losing some local species of plants or fish was (READ ITEM)?[b]

Risk level	Approve	Not approve	Not sure	No answer[c]
Unknown	31%	65%	3%	1%
1 in 100	40	51	9	0
1 in 1000	55	37	3	5
1 in 10,000	65	27	3	5
1 in 100,000	71	21	3	5
1 in 1,000,000	74	18	2	5
Unknown, but very remote	45	46	9	5

[a] Percentages are presented as weighted sample estimates. The unweighted base from which the sampling variance can be calculated is 1,273.

[b] Approvals are cumulative. Persons who approved at a risk level were not asked to approve at lower levels of risk.

[c] As a result of a programming error, those who approved at "Unknown" risk level were not asked about specific risk levels. Those omitted were recontacted to complete the risk section but we were unable to obtain responses from 5 percent of the sample, as a result. Although these are treated as "No answer," most of them would be approvals at the first risk level specified.

SOURCE: Office of Technology Assessment, 1987.

Table 3-3.—Attitudes About Environmental Uses of Genetic Engineering Under Remote Risk Conditions[a]

Q.: If there was no direct risk to humans and only very remote risks to the environment, would you approve or disapprove the environmental use of genetically engineered organisms designed to produce (READ ITEM)?

	Approve	Disapprove	Not sure
Disease-resistant crops	73%	23%	4%
Bacteria to clean oil spills	73	23	4
Frost resistant crops	70	27	3
More effective pesticides	56	40	4
Larger game fish	53	43	4

[a] Percentages are presented as weighted sample estimates. The unweighted base from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

optimism if reasonable precautions are taken by those developing, applying, and approving the new technologies. Significant groups within the population do, however, depart from these feelings. Nevertheless, a large majority (82 percent) believes that research into genetic engineering should continue, and support for this research is found in all segments of the American public (table 3-5).

This research enjoys majority support even among those who believe human cell manipulations are morally wrong (71 percent), that genetic engineering products will present serious risks (73 percent), or that it would be better if we did not know how to alter cells genetically (63 percent) (30).

A plurality (40 percent) feels that government funding for biological research should be increased. Only 10 percent of the public thinks that government funding for biological research should be cut.

Table 3-4.—Environmental Release on an Experimental Basis[a]

Q.: Do you think that environmental applications of genetically altered organisms to increase agricultural productivity or clean up environmental pollutants should be permitted on a small scale, experimental basis, or not?

Yes	82%
No	13
Not sure	4

Q.: Do you think that commercial firms should be permitted to apply genetically altered organisms on a large scale basis, if the risks of environmental danger are judged to be very small, or not?

Yes	42%
No	53
Not sure	5

Q.: Suppose your community was selected as the site to test a genetically altered organism—such as bacteria that protect strawberries from frost—where there was no direct risk to humans and a very remote potential risk to the local environment. Would you be strongly in favor, somewhat in favor, somewhat opposed, very opposed, or really not care if it was used in your community?

Strongly in favor	14%
Somewhat in favor	39
Don't care	14
Somewhat opposed	21
Strongly opposed	11
Not sure	2

[a] Percentages are presented as weighted sample estimates. The unweighted base from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

Table 3-5.—Opinions About Genetic Research and Funding

Q.: Do you think that research into genetic engineering should be continued or should be stopped?

Continued	82%
Stopped	13
Not sure	5

Q.: Do you believe that government funding for biologic research should be increased substantially, increased somewhat, remain about the same, decreased somewhat, or decreased substantially?

Increased substantially	11%
Increased somewhat	29
Remain about the same	43
Decreased somewhat	6
Decreased substantially	4
Not sure	7

[a] Percentages are presented as weighted sample estimates. The unweighted base from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

Aside from supporting research, the public recognizes another important government function associated with the development of biotechnology. A plurality (37 percent) says the government should decide whether commercial firms should be permitted to apply genetically engineered organisms on a large-scale, commercial basis. Twenty-nine percent claim this decision should be made by an external, scientific body, while only 5 percent feel voters, taxpayers, or other community-based groups should make the decision. Thirteen percent maintain the company involved should make the decision, and 4 percent find a role for industrial trade organizations (table 3-6).

Despite this relative ranking, which gives the highest degree of approval for decisionmaking to governmental agencies, the public has more confidence in university scientists than in the government. When asked whose statements they would be likely to believe about the safety of a particular application, the majority of Americans say university scientists (86 percent). Public health officials score second (82 percent), environmental groups third (71 percent), followed by governmental agencies (69 percent). In the case of conflicting statements from governmental agencies and environmental groups, 26 percent would favor the Federal agency, 63 percent place more trust in the environmental group, and 11 percent are undecided or say it would depend on the specific circumstances.

In summary, the survey found that while the public is concerned about genetic engineering in the abstract, most people approve nearly every specific environmental or therapeutic application explored in this poll. The public is sufficiently concerned about potential risks to say that strict regulation is necessary, yet a majority also agree strongly or somewhat that unjustified fears of these new technologies have seriously impeded the development of valuable new drugs and therapies, and that the risks have been exaggerated (table 3-7).

As in other areas of science and technology, the public favors the continued development and application of biotechnology because people believe the benefits will justify the risks. Strict regulation to avoid unnecessary risk is expected, but some risk is clearly acceptable if sufficient benefit is expected in return.

Table 3-6.—Who Should Decide?

Q.: Who should be responsible for deciding whether or not commercial firms should be permitted to apply genetically altered organisms on a large scale basis--the company that developed the product, an external scientific body, a government agency, an industrial trade association, or other group?

			Party affiliation		
	Total	Voters	Republican	Independent	Democrat
Government agency	37%	38%	38%	35%	38%
External scientific body	29	31	32	34	25
Company that developed product	13	12	12	8	16
Public/voters/taxpayers community	5	4	4	4	5
Industrial trade association	4	4	3	4	4
Not sure	5	5	4	5	5
All other mentions	8				

[a] Percentages are presented as weighted sample estimates. The unweighted base for the total sample from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

Table 3-7.—General Perceptions Concerning Biotechnology[a]

Q.: I will now read you a few statements. For each, please tell me whether you agree strongly, agree somewhat, disagree somewhat or disagree strongly. (READ EACH ITEM)

	Agree strongly	Agree somewhat	Disagree somewhat	Disagree strongly	Not sure
a. The potential danger from genetically altered cells and microbes is so great that strict regulations are necessary	43%	34%	14%	6%	3%
b. The risks of genetic engineering have been greatly exaggerated	15	40	27	10	8
c. It would be better if we did not know how to genetically alter cells at all	13	20	34	31	2
d. The unjustified fears of genetic engineering have seriously impeded the development of valuable new drugs and therapies	20	38	26	9	8
e. We have no business meddling with nature	26	20	31	21	2

[a]Percentages are presented as weighted sample estimates. The unweighted sample from which the sampling variance can be calculated is 1,273.

SOURCE: Office of Technology Assessment, 1987.

THE ROLE OF PUBLIC PERCEPTION IN LOCAL COMMUNITIES

Companies and researchers must be prepared to work with State and local officials and residents when a field test of a genetically altered plant, animal, or micro-organism is proposed. Without local support, proposed field tests may be delayed or canceled. To date, several proposed field tests have met with varying degrees of State and local support. Several States have or are currently considering legislation based on the perception that additional State protection or coordination is needed (see box 3-A).

The experiences of 11 communities described in this section illustrate varying degrees of local acceptance of proposed field tests, and varying degrees of State and local oversight of such experiments.

Monterey and Contra Costa Counties, California

> "It was only local concern, generated in Monterey, that opened up the issue. As with events in the nuclear industry, public opinion only becomes focused when it is in your backyard."
>
> Roger Sherwood,
> "The Monterey fallout continues,"
> *Trends in Biotechnology*, July 1986.

Advanced Genetic Sciences, Inc. (AGS) of Oakland, California received the first experimental use permit issued by the Environmental Protection Agency (EPA) for the environmental release of a genetically altered organism. The AGS permit was also the first to be revoked by EPA.

Contra Costa
Monterey

In November 1985, EPA approved the issuance of an experimental use permit to release strains of *Pseudomonas syringae* and *P. fluorescens* from which the gene for the ice-nucleation protein had been deleted. The altered bacteria (also known as Frostban) was to be applied to 2,400 strawberry plants on an 0.2-acre plot surrounded by a 49-foot vegetation-free zone in northern Salinas Valley, California.

Various individuals and nonprofit environmental organizations sought injunctive relief against EPA's issuance of an experimental use permit to AGS. The suit was dismissed in March 1986 on the grounds that the plaintiffs (Foundation on Economic Trends et al.) failed to establish the likelihood of success in showing that EPA's issuance of a permit violated the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the National Environmental Policy Act (NEPA) or the Administrative Procedure Act (14).

In January 1986, the Monterey County Board of Supervisors held a hearing—receiving testimony

Box 3-A.—State Legislative Activity

California

In 1984, the California Legislature passed Assembly Concurrent Resolution 170 "to promote the biotechnology industry, while at the same time protecting public health and safety and the environment." As a result, the Governor's Task Force on Biotechnology prepared a guide to clarify the regulatory procedures for biotechnology (26).

A bill (SB 844) introduced into the Senate on March 3, 1987, would require State regulations on the handling of biotechnologically novel organisms, making violators subject to civil and criminal penalties.

Illinois

Legislation was introduced (HB 1866) in 1987 establishing a 9-member committee to review existing Federal regulations and monitor the release of genetically engineered organisms. The bill was passed by the Legislature, but vetoed for budgetary reasons by Governor James Thompson on October 22, 1987.

New Jersey

New Jersey State bill S.1123 (introduced in the 1986 session) would find that "the citizens of the state maintain legitimate concerns about the effect that the release of genetically engineered micro-organisms into the outdoor environment may have on the health, safety and welfare of the public," and establish a 9-member commission to regulate the release of genetically engineered micro-organisms in the environment. The bill has been opposed by the Association of Biotechnology Companies (18), which is concerned that such legislation "would put New Jersey on the map as being antagonistic to the emerging biotechnology industry and thereby discouraging companies from locating their high-technology research and manufacturing jobs within the state" (19).

The bill was approved by the Senate but defeated by the State Assembly.

North Carolina

The North Carolina General Assembly approved the formation of a study commission approved by the Joint Appropriations Committee, in July 1986, to determine whether a State environmental protection agency should be formed.

Texas

HB 41, introduced during the 1987 legislative session, would have established a commission to review the adequacy of Federal and State laws governing biotechnology, and requiring State notification of releases of genetically engineered organisms. The bill was not acted upon.

Wisconsin

Two members of the Senate Agriculture, Health and Human Services Committee recommended the creation of a legislative council committee, that would consist of legislators and other interested parties, to study how the State should regulate biotechnology. This followed a 1987 report from the Department of Natural Resources that was critical of the Federal Coordinated Framework. In January 1988, a legislative subcommittee approved a proposal that would require experimenters to apply for a State permit prior to releasing any genetically altered organism into the environment.

SOURCE: Office of Technology Assessment, 1988.

from EPA, California State Departments of Health Services, Food and Agriculture, AGS, scientists, the Foundation on Economic Trends, and concerned members of the public. An ordinance banning experiments in Monterey County for 45 days was passed by the Supervisors. In February 1986, it was learned that AGS had 1 year previously injected the test bacteria into approximately 50 fruit trees on the rooftop of its headquarters building without EPA approval. In March 1986, EPA suspended the AGS experimental use permit and fined the company $20,000 on the grounds that the organism had been released prior to EPA approval and that the company had deliberately made false statements on its application. The fine was later reduced to $13,000 with an amended complaint that AGS had not provided adequate details about the testing method. In April 1986, the Monterey County supervisors, relying on their zoning authority, passed legislation banning experiments within the county for a year.

In December 1986, AGS applied to EPA and the California Department of Food and Agriculture for approval to conduct the field test in San Benito County or Contra Costa County (both in California). In February 1987, the EPA reissued an experimental use permit to AGS, and the State Department of Food and Agriculture gave its preliminary approval. In March, after receiving the approval of the Contra Costa County Board of Supervisors, AGS announced its intention to conduct the field test outside of Brentwood, a town with approximately 6,000 residents. Opponents filed a legal challenge in April, which was dismissed by a Sacramento County Superior Court judge. On April 24, 1987, the field test was carried out, even though many of the plants were uprooted by vandals just hours prior to the test. A second test on 17,500 strawberry plants commenced in December 1987.

Local reaction to the AGS proposed field test differs from the experience of other communities (described later) in several respects. The AGS proposed field test was the first to be approved by EPA, and the only one to be suspended by EPA. The proposed test site in Monterey County was also in a more populous area than the others (a situation remedied by the relocation of the proposed release site). Finally, disclosure in the media alleging that AGS had conducted a limited environmental release on its headquarters rooftop opened the company, and the environmental release issue generally, to closer public scrutiny.

Tulelake, California

> "We object to using the Tulelake area as guinea pigs for an experiment that won't benefit the area and could cause public refusal of local farm products."
>
> Joe Victrene, master, Tulelake Grange,
> during public hearing, Jan. 10, 1987.

Tulelake, an agricultural town near the California/Oregon border, was the proposed test site for the release of genetically altered *P. syringae* bacteria on a small plot of potatoes. Designed by Steven Lindow and Nickolas Panopoulos, plant pathologists at the University of California at Berkeley, the experiment involved identifying the gene responsible for ice nucleation, deleting the gene, and applying the altered bacterium to the plants. If successful, the treated potato plants might resist frost damage from temperatures as low as 23 °F.

The proposed environmental release of the ice-minus bacteria was first approved by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC) in April 1983. In September 1983, the Foundation on Economic Trends joined several other groups and individuals in filing suit to stop the experiment. The plaintiffs argued that NIH had violated the administrative requirements of the NEPA, which requires Federal officials to prepare an environmental impact statement before approving an action significantly affecting the environment. In May 1984, Judge John Sirica issued an injunction prohibiting the field test, and barring NIH from approving further experiments involving the release of engineered organisms until it assessed the environmental impacts of such tests. EPA began review of the experiment in 1984. In 1986, EPA and the U.S. Department of Agriculture (USDA) assumed regulatory authority, pursuant to the Federal Government's Coordinated Framework for the Regulation of Biotechnology.

Local opposition to the proposed field test received increased attention in 1986. In early May, field test opponents circulated a petition that garnered 450 signatures. The group, "Concerned Citizens of Tulelake," were present at a May 1986 public meeting, and then appeared before the Tulelake City Council and the Siskiyou and Modoc County Board of Supervisors seeking local governmental action against the experiment. On June 2, 1986, the Modoc County Board of Supervisors passed a legally nonbinding resolution opposing the experiment on the grounds that "the questions and fears in the minds of the public could have a serious and immediate adverse effect on the market for crops from the area."

Despite the protest, on May 13, 1986, the EPA approved the experiment and issued an experimental use permit, saying that the environmental release posed "minimal risk to public health or the environment." In July, the scientists announced that they would proceed with the experiment in early August. On August 1, opponents of the test (Californians for Responsible Toxic Management and the Foundation on Economic Trends) filed suit in Sacramento Superior Court against the University of California-Berkeley Regents and the California Department of Food and Agriculture seeking an injunction against the experiment until environmental impact studies could be done at State level. On August 4, 1986, 2 days prior to the proposed field test, Sacramento County Superior Court Judge A. Richard Backus granted an 18-day temporary restraining order. Two weeks later, the University of California agreed to halt the experiment for 1986.

Local opposition to the Tulelake field test continued at a public hearing in January 1987, when several residents, including the president of the Tulelake Growers Association and the master of Tulelake Grange, voiced opposition to the experiment, fearing crops may be boycotted by buyers. On April 29, 1987, 3 days after Advanced Genetic Sciences, Inc. began its field test of Frostban in Contra Costa County, the University of California scientists planted potato tubers treated with the ice-minus bacterium on a half-acre site at a university field station near Tulelake. On May 26, 1987, vandals uprooted approximately half of the plants being studied. Earth First!, an environmental group, claimed responsibility for the raid, which disrupted attempts to study the yields from the plants, but not attempts to study how well the bacteria established themselves on the plants (1).

The Tulelake scenario is similar to that of Monterey County. Both experiments involved proposed releases of *P. syringae*, both followed similar regulatory approval processes, and both were linked together in many media stories. While both experiments elicited opposition in their respective communities, in Tulelake it focused to a significant degree on a fear that locally grown crops would be boycotted by buyers, damaging the local economy. Although opponents of the Monterey County and Tulelake field tests went to county authorities to stop the experiments, opponents of the Tulelake field test also relied on State environmental law as the basis for their suit in Sacramento County Superior Court. In both instances, experimental plants were vandalized.

St. Charles County, Missouri

> "The Monsanto case is the third product to move through regulatory agencies and is the most controversial because the bacterium produces a poison which will kill some living things, and which may remain for some time in the soil before dying."
>
> Phillip J. Hilts, *Washington Post*, Apr. 25, 1986.

Monsanto proposed releasing an engineered microbial pesticide designed to protect the roots of corn plants against black cutworm. Although the company was prepared to proceed with the release in mid-1984, it held off until publication of proposed Federal guidelines late in the year. Monsanto then became the first company to seek EPA approval following publication of the Federal Government's Proposed Coordinated Framework for Regulation of Biotechnology. In April 1986, an EPA scientific committee recommended that the Monsanto field test should be allowed to proceed (5).

Local opposition to the Monsanto experiment became apparent when the St. Charles City Council passed a resolution in early 1986 opposing the procedure. This opposition ran counter to the test's endorsement by the 3-person St. Charles County Commission. In March 1986, the Foundation on Economic Trends petitioned EPA to deny Monsanto's permit application, citing "unresolved questions regarding the nature and magnitude of the risks and benefits involved in the Monsanto proposal." On May 8, 1986, St. Charles County officials delivered a letter to Monsanto threatening to sue if the company proceeded with the field test. The letter cited county code sections prohibiting storage or processing of anything considered harmful or potentially harmful to individuals or the environment and specifying that flood plains must be used for agricultural purposes.

Twelve days later, EPA requested additional information in support of Monsanto's application for an experimental use permit. Results of the additional tests are pending.

Middleton, Wisconsin

> "The Agracetus project has not elicited much protest, however, even from the Foundation on Economic Trends, a frequent opponent of such tests. 'We looked at it and it didn't raise the kind of fundamental questions the other tests did,' said Jeremy Rifkin, the foundation's president. Mr. Rifkin said that in general genetically modified plants pose less risk than micro-organisms because they can be contained more easily.' "
>
> —Andrew Pollock, *New York Times*, May 30, 1986.

The Agracetus Corp. (a joint venture of Cetus and W.R. Grace) proposed to insert an altered gene for disease resistance against crown gall tumors in 200 tobacco plant seedlings. The genetically modified plants were planted in a one-twentieth-acre plot in Middleton, WI. Tobacco was the experimental plant of choice because it is one of the easiest plants to engineer genetically. Agracetus received approval for the field test from the NIH's RAC, USDA, and the Wisconsin Department of Natural Resources.

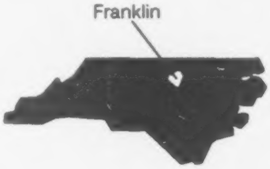
On May 30, 1986, Agracetus commenced the first authorized planting of a genetically altered crop. The *Wisconsin State Journal* noted its approval for the experiment, stating that "while it remains to be seen if the test will prove scientifically successful, it is already a winner from the regulatory point of view." The editorial noted that regulatory approval was "by the book," and that the release site was situated away from roads or people.

Unlike the proposed releases of genetically altered organisms in Monterey County, Tulelake, and St. Charles County, the Agracetus experiment involved the introduction of a genetically altered plant. Local newspaper accounts of the Agracetus experiment talked of the potential economic gain to be realized should crops be made resistant to crown gall tumors.

Franklin County, North Carolina

> "Ciba-Geigy officials have informed state and local officials of their plans. 'I think they have done a responsible job,' said Earl R. MacCormac, science advisor to Gov. James G. Martin. 'I feel real good about it,' added Ronald W. Goswick, chairman of the Franklin County Board of Commissioners."
>
> —Monte Basgall, Raleigh, *N.C. News & Observer*, June 18, 1986.

The Agricultural Division of Ciba-Geigy Corp., Greensboro, NC, proposed conducting a field test of a tobacco plant that had been genetically altered to resist atrazine, a herbicide used to control weeds in corn, sorghum, and other crops. Certain crops, including tobacco and soybeans, are susceptible to atrazine. These crops can be injured when planted in some soil types the year after a tolerant crop treated with atrazine was grown there, since residual atrazine persists in the soil through the period of crop rotation.



Ciba-Geigy applied to USDA for approval of the field test. The North Carolina Department of Agriculture asked that USDA regulate the test because no State guidelines existed for handling such research.

USDA approved the field test in July 1986. The North Carolina legislature subsequently approved funding for a study commission to determine whether control of State environmental responsibilities needed to be consolidated.

Mississippi and Florida

> "The Rohm and Haas Company of Philadelphia, one of the world's largest producers of chemicals, announced Wednesday that the U.S. Agriculture Department had approved the world's first field test of genetically engineered caterpillar-resistant plants."
>
> —Associated Press, Aug. 28, 1986.

Rohm and Haas developed tobacco plants altered by the addition of a single gene from the bacterium *Bacillus thuringiensis*. The altered plants, developed by a Belgian company for Rohm & Haas, were designed to be resistant to leaf-eating caterpillars.

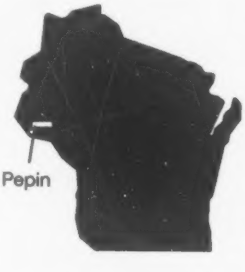
In June 1986, Rohm and Haas announced that it had voluntarily applied to USDA for permission to field test the tobacco plants at company-owned research farms near Cleveland, MS and Homestead, FL. USDA issued an opinion letter in August 1986 stating that the "genetically engineered tobacco plants are not plant pests" (51 Fed. Reg. 32237).

Prior to publicly announcing its proposed field test, Rohm and Haas provided information to appropriate Federal, State, and local representatives for both test sites. This was followed up by two presentations for the local public and media on the day the proposed field tests were announced (22). Later, presentations were made to other interested groups, including the Central Mississippi Chapter of the Sierra Club. According to one member of the Sierra chapter, the presentation elicited no grave concerns, leaving the impression that the experiment seems valid and safe (3).

Pepin County, Wisconsin

> "Does this test pose a high risk? No. EPA believes that this field test poses little or no risk for several reasons. The genetically engineered strains are expected to be no different than the naturally occurring strains, except for the enhancement of a preexisting trait (the ability to fix nitrogen from the air to the soil). This is significant because the naturally occurring strains have been the most extensively studied microorganisms in agriculture (they have been studied for nearly 100 years) and have shown no significant adverse effects."
>
> —Environmental Protection Agency, "Note to Correspondents" Fact Sheet, Apr. 29, 1987.

BioTechnica International, Inc. of Cambridge, MA, proposed a field test of genetically engineered strains of *Rhizobium meliloti*, a bacterium involved in nitrogen fixation in alfalfa. *Rhizobium* is a genus of bacteria commonly used in agriculture, with various strains being sold commercially to increase the yields of legume crops. In its proposal to EPA and USDA, filed on February 6, 1987, BioTechnica noted that about 80 percent of the U.S. alfalfa acreage and 15 to 25 percent of the soybean acreage are inoculated with nongenetically engineered rhizobia-based products. The genetically engineered *Rhizobium* converts atmospheric nitrogen at an increased level, resulting in increased alfalfa yields of up to 17 percent in greenhouse studies by BioTechnica.



The BioTechnica proposal was the first application under the Toxic Substances Control Act (TSCA) subject to the EPA biotechnology policy published in the Federal Register on June 26, 1986. BioTechnica's application was filed with EPA in February 1987.

The proposed field test site is BioTechnica's Chippewa Agricultural Station near Arkansaw, WI, an unincorporated town in the Waterville Township of Pepin County. The area of the proposed test site is lightly populated and far from urban areas. The total population of Pepin County is approximately 7,500, of whom approximately

Photo credit: Kevin O'Connor

Pepin County, Wisconsin

1,000 reside in Waterville Township. The station, located about 30 miles from Eau Claire, WI, and 75 miles from St. Paul, MN, is a 360-acre farm, with less than 5 acres of the site designated for the field test.

The company produced a brochure designed to provide local residents with information regarding the proposed field test. The brochure addresses 21 questions that have been raised during the course of meetings between BioTechnica officials and local residents (see box 3-B). Another brochure, a nontechnical description of the field test, was also produced.

EPA gave tentative approval for the field test in May 1987, but delayed the experiment in order to extend the public comment period and to review how the test would be monitored. In July 1987, a hearing sponsored by the Wisconsin Senate Agriculture, Health, and Human Services Committee was held. Although little opposition was voiced regarding BioTechnica's proposed test, residents did express concerns about potential future tests (12). In August 1987, BioTechnica announced a postponement of the field test following EPA concerns regarding monitoring of the altered bacteria. BioTechnica concluded that the altered bacteria could not be distinguished from other common bacteria present in the soil. The company plans to develop an alternate monitoring plan, and expects to obtain final EPA approval in 1988 (15).

Box 3-B.—Pepin County and Biotechnology: 21 Questions and Answers

1. What is the Chippewa Agricultural Station? The Chippewa Agricultural Station was established in January 1987 in Arkansaw (Waterville Township), Pepin County, Wisconsin. It is a wholly owned subsidiary of BioTechnica International, Inc. of Cambridge, Massachusetts. Formed in 1981, BTI is a biotechnology research and development company with commercial operations in agriculture and dental diagnostics. Its stock is traded on the national over-the-counter market. The new agricultural station is BTI's first expansion effort outside its home state and its first agricultural station. The station will be used for conventional farming operations, as well as for agricultural research.

2. Why did BioTechnica International choose Wisconsin? Wisconsin is the Nation's leading dairy State, and it is also the number one producer of alfalfa. The State has been receptive to new advances in business and agriculture. Wisconsin has a sound policy for environmental protection which BTI supports. Field tests of biotechnology products have already been successfully conducted within the State. The University of Wisconsin offers outstanding research and academic expertise, and its Biotechnology Center in Madison is a highly effective channel for communications between the academic and industrial communities and public officials.

3. Why was Pepin County, one of the smallest counties in Wisconsin, chosen for BioTechnica International's research station? Pepin County was selected for BTI's first agricultural research station for several outstanding reasons:

- Wisconsin is the nation's top producer of alfalfa, the plant which will be field tested at the new site.
- The site is close to the University of Wisconsin's Marshfield Research Station, where BTI is currently conducting field tests of a new conventional silage additive. There is a possibility that this research effort will be moved to Pepin County.

- The area has excellent rainfall.
- BTI was able to buy the 360-acre site at a very reasonable price.
- An irrigation system was already in place on the farm.
- The soil at the farm is a sandy loam and is uniform throughout the 360 acres, almost unheard-of benefits for an agricultural research station.
- The land is exceptionally flat, minimizing runoff.
- The site is 170 feet above the aquifer, making it virtually impossible for groundwater contamination from the field tests to take place.
- The site is protected by hills and trees, and is an infrequent host to severe winds and tornados.
- The site lies within the 110-day corn-maturity zone and Group 2 soybean zone which make it a very favorable crop-growing climate.
- The Chippewa Agricultural Station is less than 1 mile from the home of the farm's superintendent.

4. Will the entire research farm be used for this field test? Absolutely not. Less than 5 acres, or slightly more than 1 percent, of the 360 acres will be used in the field test this year. Conventional crops such as alfalfa, soybeans, corn, beans, tobacco, and rapeseed are being considered for planting in the spring and summer of 1987.

5. Why is your company planning field tests at the farm? Both laboratory and greenhouse tests have shown that BTI's genetically engineered *Rhizobium meliloti* increases alfalfa yields by as much as 20 percent. Long before any agricultural product, conventional or genetically engineered, goes to market it must first be field tested to determine if it is effective under the actual growing conditions encountered in the field.

6. What is *Rhizobium meliloti* anyway? Rhizobia are naturally occurring bacteria that exist in the soil. The rhizobia have a symbiotic relationship with legumes such as alfalfa, soybeans, peas, and beans. Rhizobia attach themselves to the plant's roots and establish root nodules where they live. The plant gives the rhizobia a home and a food source; the rhizobia, in turn, convert atmospheric nitrogen into a form which the plant can use. *Rhizobium meliloti* is the species that naturally associates with alfalfa.

7. How long have rhizobia been around? Farmers have been aware of this unique relationship and have taken advantage of it in rotational farming for thousands of years. Rhizobia have been commercially available in the U.S. since the 1890s and are widely used today by farmers and home gardeners. The largest producer today of commercial rhizobia inoculants is located in Milwaukee.

8. So what did you do to the rhizobia? Very simply, our scientists "souped up" the bacteria's ability to supply nitrogen to alfalfa.

9. How did they do that? Through a laborious research process that took several years, our scientists identified the genes that are responsible for supplying nitrogen to plants. Using a surgically precise process called gene splicing, they were able to alter certain genes so that their nitrogen-fixing, or nitrogen-gathering ability was greatly increased.

10. Is this some kind of "Super Bug"? It definitely is an improvement of a naturally occurring bacterium, but by no means can it be considered a dangerous "Super Bug." Many of the strains now used commercially have been carefully selected by the USDA for improved performance. BTI's work is a natural outgrowth of such efforts to provide better products to farmers.

11. How do you know it isn't dangerous? Rhizobia are perhaps the best-studied bacteria in agriculture. They have a specific function in nature and that is to gather nitrogen for leguminous plants. In over 90 years of commercial use, they have never been found to be dangerous to plants, animals, or man, nor have they been known to be threats to the environment. Furthermore, BTI has tested its new strains in the laboratory and has shown them to behave the same as their natural counterparts.

12. Couldn't a whole field of these new organisms deplete the nitrogen supply? They couldn't even put a dent in it. First, the atmosphere is about 80 percent nitrogen, with over 33,000 tons of nitrogen above every acre of land and water on earth. Naturally occurring rhizobia in an acre of alfalfa gather from 100 to 200 lbs. of nitrogen per year. BTI will be very pleased if its strains do twice as well. Secondly, Mother Nature is full of checks and balances as the nitrogen cycle proves: nitrogen taken from the atmosphere is ultimately returned to the atmosphere.

13. Won't higher yielding plants drain more nutrients from the soil? Quite the opposite. Some of the gathered nitrogen will leak into the soil and will be available to crops grown in rotation with legumes. That's why farmers apply less nitrogen fertilizer to corn planted the year after alfalfa or soybeans. Since BTI's strains will gather more nitrogen, it is expected that they will leave more nitrogen behind, thus further enriching the soil.

14. Can this improved version hurt cattle and eventually humans? Remember, the only alteration to the rhizobia will be to improve their natural nitrogen-gathering ability. Based on all scientific evidence we have, there is nothing to indicate that there could be any adverse effects on cattle or man. The nitrogen-gathering process occurs only in the roots which are not harvested. Cattle don't eat alfalfa roots.

15. Can this new version affect other plants? No. Rhizobia function only in association with leguminous plants. And, there are certain types of legumes for each rhizobial species. For instance, *R. meliloti* for alfalfa, *R. japonicum* for soybeans, etc. BTI's changes to *R. meliloti* will not cause it to affect any other crop species.

16. How far can these little creatures travel on their own? Not very far at all. Their entire range of motility is only about two-tenths of an inch per day, or, about 1½ feet in a 100-day growing season.

17. Can they be blown away by the wind? They live under the soil, so it would take a pretty strong wind to blow them away. The test site is well shielded from the wind. But the *R. meliloti* die without moisture and when exposed to the ultraviolet light of the sun. So, if they were blown away, their chances of survival would be nil.

18. Suppose a hard rain came along, and some of your topsoil washed away with your new strains and got into some streams and lakes. Could they cause a danger to fish and aquatic plants? It is highly unlikely that the new strains could survive for any length of time in the water, since water lacks many of the nutrients Rhizobia need to grow. They aren't toxic, and the levels of ammonia they produce could not possibly be high enough to have any adverse effect on aquatic plants.

19. It sounds safe, but has it been approved by the government? Before the field testing can begin, our application to field test must be approved by the United States Department of Agriculture and the Environmental Protection Agency. In addition, our application will have undergone very close scrutiny by Wisconsin's Department of Agriculture, Trade and Consumer Protection, and by the Department of Natural Resources. It goes without saying that BTI welcomes and appreciates the approval of both the county leaders and the citizens of Pepin County.

20. With so many crop surpluses being reported, why aim for yield increase? It is true that there are many crop surpluses in the world today. With this particular field test, we are looking to achieve higher yield increases in the 15-20 percent range. But the value to farmers comes in gains in productivity. If our tests prove successful and we market this new product, farmers will be able to produce the same *or more* alfalfa on less land at lower unit cost.

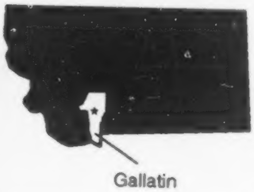
21. Once these tests have been ended, will your company pack its bags and move somewhere else? Not very likely. We have invested a good amount of money in the farm, its rehabilitation, its new buildings, and in its equipment. Chippewa employees are all residents of Pepin County, and all of our farm purchases will be made through area merchants whenever possible. We have made the agricultural market a primary objective for BTI's growth and development as a leader in the biotechnology field. Pepin County and the Chippewa Agricultural Station will play a major part in the long range progress and growth of BioTechnica International, Inc.

SOURCE: BioTechnica International, 1987.

Bozeman, Montana

> "We can sit and talk elm disease, or we can do something about it. I choose to do something about it."
>
> —Gary Strobel, Aug. 13, 1987.

Gary Strobel, a professor of plant pathology at Montana State University, injected 14 trees with a fungus that causes Dutch elm disease. Half of the trees had previously been injected with genetically altered bacteria designed to fight the disease. The experiment, carried out without Federal Government approval and without the knowledge of the university's biosafety committee, was initiated in June 1987. Two months later, the uninjected trees were dead; the injected trees were still alive. Overshadowing the scientific data, however, was the publicity aroused by Strobel's experiment as portrayed in headlines such as "Genetic Engineering Rules: The Making of a Monster" (34).



The 14 trees were located on the Montana State campus. Strobel initiated the field test in June in order to obtain results during the current year's growing season. When EPA contacted him in July 1987 requesting further information on the test, Strobel notified the agency that the experiment had already begun. In August 1987, Strobel notified his university's biosafety committee of the details of the field test. Shortly thereafter, the biosafety committee recommended that the trees be uprooted and burned. EPA and the university president reprimanded Strobel, and Strobel himself cut down the trees and terminated the experiment in early September.

The Montana State experience differed from the others in that the researcher had not obtained permission from either the Federal Government or the university prior to carrying out the experiment. At first, Strobel called his action "civil disobedience," a characterization he withdrew 2 weeks later. The EPA sent Strobel a notice of warning, telling Strobel that for a period of 1 year, any application for a proposed test would have to be "cosponsored by the university, a colleague or some other responsible party" (33). A written warning was the most stringent legal option available to EPA under FIFRA penalty provisions (7 U.S.C. 136(a)(2)). A Montana State University administrative review panel recommended that the university's administration issue a formal reprimand. In January 1988, NIH decided that Strobel violated no NIH Guidelines in his experiments.

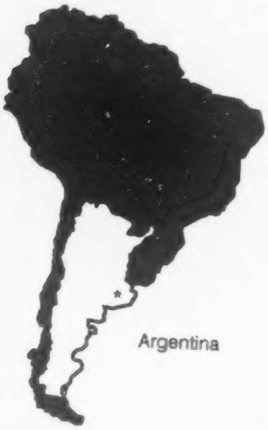
Photo credit: Gary Strobel

Gary Strobel and injected elm trees at Montana State University. Inoculated experimental tree visible on right. Unprotected control tree, dead, on left.

Unhappiness with the Federal regulatory framework was voiced by Strobel, and by Montana State University President William Teitz, who in reprimanding Strobel also complained of the "tangled interpretations, definitions, procedures, exceptions, inclusions, and classifications that dominate today's biotechnical research."

Argentina

> "There is a lesson to be drawn from this which I wish to pass on to my scientific colleagues: extreme caution is to be observed in conducting cooperative programs with organizations and scientists who have political motives which intrude upon even the most straightforward attempts to conduct scientific research for the benefit of humanity."
>
> —Hilary Koprowski, Director, Wistar Institute



The Wistar Institute of Philadelphia produced a genetically engineered rabies vaccine, which it provided to the Pan American Health Organization (PAHO) for field testing in Argentina. In July 1986, 20 cattle were inoculated at a PAHO agriculture station in Azul, Argentina (approximately 180 miles south of Buenos Aires). In September 1986, the Argentine government learned of the field test through a letter written by an Argentine trainee. The government barred further tests, claiming that the vaccine posed a health threat. On November 11, 1986, the *New York Times* reported that Wistar conducted tests the previous summer without obtaining approval from either the Argentine or United States governments. In December 1986, NIH sought written assurance from Wistar that no Federal funds were used to test the rabies vaccine in Argentina. Wistar replied that although the rabies vaccine research program received Federal funding, $100,000 for the Argentina field test came from private sources. In January 1987, NIH announced that the experiment did not violate NIH guidelines.

Criticism of the Wistar-PAHO experiment was voiced editorially by the *New York Times* (29) and *Los Angeles Times* (6) as well as by 134 Argentine scientists, who alleged violations of ethical, ecological, and safety rules (16). The director of the Wistar Institute maintained that media accounts ignored the results of the experiment and that the vaccine was both efficient and safe; that Wistar merely provided the vaccine to PAHO, anticipating that the health organization would obtain any necessary governmental approvals in Argentina; and that representatives of an Argentine scientific organization approached Wistar in 1984 proposing to conduct the same trial undertaken by PAHO.

New Zealand

Another overseas test of a genetically engineered vaccine (Bacteroides nodosus) involved researchers at Oregon State University, who, in May 1985, obtained permission from the New Zealand Ministry of Agriculture and Fisheries to import a vaccinia virus. The researchers inoculated 37 calves, 16 chickens, and 4 sheep near Wellington, New Zealand, beginning in April 1986.

New
Zealand

A *Los Angeles Times* editorial noted that "unlike the Argentine affair . . . the Oregon State people told the government of New Zealand what they intended to do" (6). In November 1986, the Foundation on Economic Trends announced it would ask USDA and other Federal agencies to investigate the New Zealand experiment and determine whether any United States laws were broken.

Great Britain

> "A cabbage patch somewhere in Britain is the unlikely venue for a world first. Since last month, the patch has been home for a collection of caterpillars that have been infected with a unique virus which does not occur naturally. The experiment may help virologists to engineer safe, artificial viruses that kill pests before they can destroy crops."
>
> —Steve Conner, *New Scientist*, Oct. 16, 1986.

Researchers at the Institute of Virology in Oxford conducted the world's first release of a genetically engineered virus when infected caterpillars were released in September 1986. The virus was engineered to contain a genetic marker so it could be tracked. The goal of the experiment was to evaluate survival and dispersal of the virus in the environment. If the experiment is successful, the researchers plan to introduce other properties into the organism, with a long-term goal of developing custom-designed viral insecticides (2).

Great Britain

The Oxford researchers consulted with the United Kingdom Advisory Committee on Genetic Manipulation; the Nature Conservancy Council; the Ministry of Agriculture, Fisheries and Food; and the Department of the Environment prior to the environmental release. In the European Parliament, news of the U.K. experiment was met with disapproval by representatives of the Green Party, who have opposed environmental release of genetically altered organisms.

THE EXISTING REGULATORY FRAMEWORK

The development of recombinant DNA techniques during the 1970s raised concerns about potential hazards posed by the new technologies. Recognizing a need to establish consensus, scientists became involved in discussing recombinant DNA technology and its potential risks. The International Conference on Recombinant DNA Molecules (better known as the Asilomar Conference) convened 140 scientists in February 1975 to address self-regulation of research involving recombinant DNA technology until its safety could be assured. Recommendations were issued assigning risk categories to various recombinant DNA experiments and containment levels for each (28).

Federal regulation of genetically altered organisms began in 1976, when NIH adopted "Guidelines for Research Involving Recombinant DNA Molecules." These stringent guidelines established containment standards and review procedures to be applied by Institutional Biosafety Committees at each institution receiving Federal support for research (31). The guidelines were modified and relaxed several times as more became known about the safety of various organisms and technologies.

The NIH Recombinant DNA Advisory Committee was the primary Federal entity for reviewing and monitoring recombinant DNA research until 1984, when its oversight of field tests was challenged by a lawsuit alleging that NIH had violated provisions of the National Environmental Policy Act (13). This act requires all Federal agencies to prepare an analysis prior to any action that may significantly alter the environment.

In 1984, the White House Office of Science and Technology Policy (OSTP) published a Proposed Coordinated Framework for the Regulation of Biotechnology (11) in order to ensure the safety of biotechnology research and products. This document proposed policies for Federal agencies responsible for reviewing the research and products of biotechnology. It also proposed the establishment of a new, centralized advisory committee within the Department of Health and Human Services (DHHS) to coordinate responses to scientific questions raised by applications received by the various Federal agencies.

Following a period for public comment, OSTP decided against establishing a committee within

DHHS. Instead, a Biotechnology Science Coordinating Committee (BSCC) was formed "to monitor the changing scene of biotechnology and serve as a means of identifying potential gaps in regulation in a timely fashion, making appropriate recommendations for either administrative or legislative action." (50 Fed. Reg. 47174). In the same notice, OSTP published an index of laws conferring authority that could be used to ensure the safety of biotechnology-related products. Many elements of the Proposed Coordinated Framework were incorporated into the Coordinated Framework published by OSTP on June 26, 1986 (51 Fed. Reg. 23301).

Table 3-8.—Agencies Responsible for Approval of Commercial Biotechnology Products

Biotechnology products	Responsible agencies
Foods/food additives	FDA*, FSIS[a]
Human drugs, medical devices, and biologics	FDA
Animal drugs	FDA
Animal biologics	APHIS
Other contained uses	EPA
Plants and animals	APHIS,[b] FSIS,[a] FDA[c]
Pesticide micro-organisms released in the environment	
All	EPA,[d] APHIS[b]
Other uses (micro-organisms):	
Intergenetic combination	EPA,[d] APHIS[b]
Intrageneric combination:	
Pathogenic source organism	
1. Agricultural use	APHIS
2. Non-agricultural use	EPA,[d] APHIS[b]
No pathogenic source organisms	EPA Report
Nonengineered pathogens:	
1. Agricultural use	APHIS
2. Non-agricultural use	EPA,[d] APHIS[b]
Nonengineered nonpathogens	EPA Report

*Designates lead agency where jurisdictions may overlap; FDA, Food and Drug Administration.

[a] FSIS, Food Safety and Inspection Service, under the Assistant Secretary of Agricultural for Marketing and Inspection Services is responsible for food use.

[b] APHIS, Animal and Plant Health Inspection Service, is involved when the micro-organism is plant pest, animal pathogen, or regulated article requiring a permit.

[c] FDA is involved when in relation to a food use.

[d] EPA requirement will only apply to environmental release under a "significant new use rule" that EPA intends to propose.

SOURCE: 51 Fed. Reg. 23339.

Coordinated Framework for Regulation of Biotechnology

The Coordinated Framework includes separate descriptions of the **regulatory policies** of the FDA, EPA, Occupational Safety and Health Administration (OSHA), and USDA; and the **research policies** of NIH, the National Science Foundation (NSF), EPA, and USDA.

The Coordinated Framework mandates both the agencies responsible for approving commercial biotechnology products (table 3-8) and the jurisdiction for biotechnology research proposals (table 3-9).Where jurisdiction overlaps, a lead agency is designated. The goal is to operate in an integrated and coordinated fashion fo cover the full range of plants, animals and micro-organisms derived by the new genetic engineering techniques.

FDA proposed no new procedures for regulating biotechnology products, instead relying on existing authority for approving drugs, human biologics, animal food additives and drugs, and medical devices. The FDA review relies on "scientific evaluation of products, and not . . . *a priori* assumptions about certain processes" and "is conducted in light of the intended use of the product on a case-by-case basis" (51 Fed. Reg. at 23309).

EPA addressed regulation of microbial products subject to two Federal statutes: the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act. EPA's review under FIFRA places "particular emphasis on small-scale field testing of genetically engineered, nonindigenous, and pathogenic microbial pesticides" (51 Fed. Reg. 23313), while TSCA provides EPA authority to regulate any organic or inorganic substance of a particular molecular identity, including any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature. Under FIFRA, all field tests of genetically altered organisms require an experimental use permit. TSCA requires a manufacturer to adhere to premanufacturing notice requirements (see box 3-C).

USDA stated that "agriculture and forestry products developed by biotechnology will not differ fundamentally from conventional products and the existing regulatory framework is adequate to regulate biotechnology" (51 Fed. Reg. at 23336). The USDA policy statement listed nine statutes considered most relevant to biotechnology applications (table 3-10).

Of primary interest is USDA's regulation of "plant pests"—any living stage of any insects, mites, nematodes, slugs, snails, protozoa, or other inver-

Table 3-9.—Jurisdiction for Biotechnology Research Proposals

Proposed research	Responsible agencies
Contained research, no release in environment:	
1. Federally funded	Funding agency[a]
2. Non-federally funded	NIH or S&E voluntary review, APHIS[b]
Foods/food additives, human drugs, medical devices biologics, animal drugs:	
1. Federally funded	FDA,[c] NIH guidelines and review
2. Non-federally funded	FDA,[c] NIH voluntary review
Plants, animals and animal biologics:	
1. Federally funded	Funding agency,[a] APHIS[b]
2. Non-federally funded	APHIS,[b] S&E voluntary review
Pesticide micro-organisms:	
Genetically engineered:	
Intergeneric	EPA,[d] APHIS,[b] S&E voluntary review
Pathogenic intrageneric	EPA,[d] APHIS,[b] S&E voluntary review
Intrageneric nonpathogen	EPA,[d] S&E voluntary review
Nonengineered:	
Nonindigenous pathogens	EPA,[d] APHIS
Indigenous pathogens	EPA,[d] APHIS
Nonindigenous nonpathogen	EPA
Other uses (micro-organisms) released in the environment:	
Genetically engineered:	
Intergeneric organisms:	
1. Federally funded	Funding agency,[a] APHIS,[b] EPA[d]
2. Commercially funded	EPA, APHIS, S&E voluntary review
Intrageneric organisms:	
Pathogenic source organism:	
1. Federally funded	Funding agency,[a] APHIS,[b] EPA[d]
2. Commercially funding	APHIS,[b] EPA[d] (if non-agricultural use)
Intrageneric combination:	
No pathogenic source organisms	EPA report
Nonengineered	EPA report,* APHIS[b]

*Designates lead agency where jurisdictions may overlap.

[a] Review and approval of research protocols conducted by NIH, S&E, or NSF.

[b] EPA jurisdiction for research on a plot greater than 10 acres.

[c] APHIS issues permits for the importation and domestic shipment of certain plants and animals, plant pests and animal pathogens, and for the shipment or release in the environment of regulated articles.

[d] EPA reviews federally funded environmental research only when it is for commercial purposes.

KEY: APHIS: Animal and Plant Health Inspection Service; EPA: Environmental Protection Agency; NIH: National Institutes of Health; S&E: United States Department of Agriculture Science and Education.

SOURCE: 51 Fed. Reg. 23305.

tebrate animals, bacteria, fungi, or parasitic plants or reproductive parts thereof, viruses, or any organisms similar to or allied with any of the forgoing, or any infectious substances, which can directly or indirectly injure or cause disease or damage in any plants or parts thereof, or any processed, manufactured, or other products of plants" (7 U.S.C. 150aa(c)).

USDA subsequently issued a final rule on the "introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests" (52 Fed. Reg. 22891). The rule sets forth procedures for obtaining a permit prior to the introduction of organisms and products that present actual or potential plant pest risks. The final rule also mandates State notification and review of permits in addition to Federal review.

Because many applications of genetically engineered organisms in the environment will be agricultural, USDA is placed in a dual role of regulating the technology while attempting to fulfill its statutory mandate "to procure, propagate, and distribute among the people new and valuable seeds and plants" (7 U.S.C. 2201). In addition, instances are likely to arise where micro-organisms that are not intended for agricultural purposes could still represent a plant pest. The Coordinated Framework addresses this issue, laying out USDA and EPA jurisdictional agreements whereby both agencies will "perform independent reviews, focusing on independent objectives" (51 Fed. Reg. 23359). EPA will review pursuant to TSCA or FIFRA, while USDA will review pursuant to the plant pest statute.

Table 3-10.—Statutes Applicable to USDA-Regulated Biotechnology

Virus-Serum Toxic Act (21 U.S.C. 151-158)
Federal Plant Pest Act (7 U.S.C. 150aa-150jj)
Plant Quarantine Act (7 U.S.C. 151-164, 166, 167)
Organic Act (7 U.S.C. 147a)
Federal Noxious Weed Act (7 U.S.C. 2801 et seq.)
Federal Seed Act (7 U.S.C. 551 et seq.)
Plant Variety Protection Act (7 U.S.C. 2321 et seq.)
Federal Meat Inspection Act (21 U.S.C. 601 et seq.)
Poultry Products Information Act (21 U.S.C. 451 et seq.)

SOURCE: 51 Fed. Reg. 23339.

Box 3-C.—EPA's Statutory Mandate: FIFRA and TSCA

Under the Coordinated Framework for Regulation of Biotechnology, the Environmental Protection Agency is addressing certain microbial products under two statutes: the Federal Insecticide, Fungicide, and Rodenticide Act; and the Toxic Substances Control Act.

Federal Insecticide, Fungicide, and Rodenticide Act

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was enacted in 1972 (PL 92-516), bringing under one statute various Federal initiatives that had been in effect as far back as 1910. FIFRA regulates the use and safety of approximately 1 billion pounds of pesticide products produced and sold annually in the United States. Approximately 70 percent of the $6 billion worth of such products are herbicides and agricultural chemicals (4).

FIFRA mandates the registration of pest control products and defined "economic poisons" with the EPA prior to the production or sale of such product. In order to register a product, an applicant must submit complete data on the product as provided in the statute (7 USC §136a). Any person may apply for an experimental use permit for a pesticide. Such a permit can be issued only if it is determined "that the applicant needs such permit in order to accumulate information necessary to register a pesticide. . ."(7 USC §136c)

Civil penalties for violations of FIFRA vary, depending on whether or not the violator is considered to be a private applicator. A private applicator must receive a written warning for a first offense. Subsequent violations can result in a fine of $1,000 for each violation. Other registrants can be assessed $5,000 for each offense, including the first offense.

Toxic Substances Control Act

The Toxic Substances Control Act (TSCA) (PL 94-469) was enacted by Congress in 1976. In contrast to other environmental statutes specifically regulating the quality of water, air, or natural resources, TSCA gave EPA broad authority to regulate "chemical substances and mixtures." Such substances and mixtures include "any organic or inorganic substance of a particular molecular identity, including any combination of such substances occurring in whole or in part as a result of a chemical reaction, or occurring in nature, and any element or uncombined element; statutory exceptions to this definition include pesticides (as defined in FIFRA, above), tobacco, special nuclear material, food, food additive, or drug. TSCA, therefore, is not designed only to regulate toxics, but also the large number of chemical substances and mixtures to which human beings and the environment are exposed each year.

Under TSCA, the manufacturer of a new chemical must submit to EPA a premanufacture notice (PMN) that describes test data relating to the identity, use, amount, chemical identity, disposal, etc. EPA then has 90 days to consider the notice and decide whether to approve production. TSCA allows EPA to ask for additional data, and to limit or ban production.

TSCA's civil penalties are harsher than those under FIFRA: up to $25,000 for each violation, with each day a violation continues constituting a separate violation.

BioTechnica's proposed field test in Pepin County, Wisconsin (see page 54) represents the first time TSCA has been used to regulate the release of a genetically engineered organism in the environment. Proponents of EPA use of TSCA contend that the statute is well-suited for evaluating the risks of field tests on a case-by-case basis. Critics contend that the 90-day review period is too short to determine the risks of a particular experiment, and that the decision-making process within EPA could prevent meaningful, accountable, pre-release screening (17).

OSHA stated that its authority under the Occupational Safety and Health Act of 1970 (29 U.S.C. et seq.) is sufficient to protect employees in the field of biotechnology, and that no further regulation is necessary.

Challenges Facing Regulators

The Coordinated Framework noted "that future scientific developments will lead to further refinements" of the regulations (51 Fed. Reg. at 23303). Several challenges face regulators as they work with the new Framework:

- **Definitions:** The Coordinated Framework provided definitions for "intergeneric organism" (i.e. a new organism) and for "pathogen." The OSTP notice makes clear that "[t]hese definitions are critical . . . for the regulation of biotechnology because they establish the types of the organisms subject to certain kinds of review" (51 Fed. Reg. 23302). NSF, FDA, and USDA announced that certain definitions "may be ambiguous" (51 Fed. Reg 44397). In addition, the BSCC "is attempting to define what constitutes 'release into the environment'." Release into the environment, "for the time being, will have somewhat varying definitions for the regulatory and research review of the different agencies" (51 Fed. Reg. 23307). In October 1986, an NIH Committee reviewing allegations surrounding an alleged field test of a pseudorabies vaccine noted that "we found ambiguities in the NIH Guidelines, both in regard to whether the pseudorabies vaccine used in the field test consisted of 'recombinant DNA molecules,' and whether the field test constituted 'deliberate release into the environment' " (32).
- **Risk Assessment and Management:** The continuing need to protect the environment and public health requires a balancing of the known risks of existing technologies and the potential risks of new technologies against the benefits derived from these technologies. Because the risks involved in most proposed releases of genetically engineered organisms into the environment cannot be measured precisely, there will be some uncertainty in determining the safety of proposed field tests.
- **The Need To Promote a Favorable Economic Climate for Research and Product Development.** Excessive regulation will make it difficult for biotechnology-related research projects to move from the controlled environment of the laboratory to the field. Initially strict regulation of recombinant DNA research, through NIH guidelines, was revised and relaxed as increased scientific knowledge revealed the safety of various applications. Decreased regulation of genetically engineered organisms in the environment may also be possible if warranted by scientific developments. If proposed field tests are severely restricted, curtailed, or delayed, researchers may conduct research and product development in other countries that are more hospitable to such technology.
- **Assurance That Regulation of One Type of Product Does Not Hinder Development of Other Products:** The new Framework provides several Federal agencies with jurisdiction over a wide range of research and products (e.g., food, drugs, pesticides, vaccines, and medical technologies). Some agencies may regulate this new technology better than others. Mistakes made in regulating research or products could erode public confidence in the entire Coordinated Framework, which could in turn lead to inconsistent regulatory review of proposed planned introductions of genetically engineered organisms.
- **Jurisdiction of Federal Agencies Regulating Biotechnology:** Agencies need to adjust to the integrated Framework, which establishes a lead agency for those instances where regulatory oversight or review is to be performed by more than one agency (see tables 3-8 and 3-9). Environmental concerns, for example, fall under the direct mandate of EPA. All Federal agencies, however, must prepare an environmental analysis for major actions significantly affecting the quality of the human environment.
- **The Legality, Applicability, and Scope of Current Statutes To Regulate the Release of Genetically Engineered Organisms:** The Coordinated Framework is predicated on the use of existing statutes (e.g., TSCA and FIFRA) to handle emerging issues in the regulation of biotechnology. The applicability (e.g., use

of a conventional chemical statute to regulate genetically engineered organisms) of such statutes may be challenged in court, as may the scope and legality of other statutes. In addition, each statute relied upon presents administrative law issues that could result in court cases.

- **Promotion v. Regulation:** Two Federal agencies—NIH and USDA—are charged with regulating biotechnology research and development while at the same time having statutory mandates to promote research and product development. NIH promotes and funds much of the nation's biomedical research pursuant to the Public Health Act, while at the same time regulating that research, including biotechnology research. USDA is mandated to procure, propagate, and distribute new and valuable seeds and plants; at the same time, it must regulate and potentially curtail new products through the application of several statutes designed to eradicate potential problems (e.g., plant pests).
- **Consistent Penalties for Violators:** Because existing statutes are being used to regulate biotechnology, varying penalties can result. For example, the two statutes relied upon by EPA (TSCA and FIFRA) carry different penalties. As a result, penalties could merely reflect the statute employed, not the actual severity of the civil or criminal act.
- **The Role of State and Local Governments in Regulating Biotechnology and Environmental Release of Genetically Engineered Organisms:** State environmental authority and county zoning and land use ordinances have played an important role in several proposed field tests and could play an increasing role in future tests. Several States are considering regulations governing the release of genetically engineered organisms in the environment. Where Federal and State Governments claim subject matter authority over such releases, the issue of Federal preemption of State action could arise.
- **Public Perceptions:** The environmental applications of genetically engineered organisms will be affected considerably by public opinion, particularly in communities that host the early field tests. Ultimately, any applications approved for general use will feel the weight of public opinion. Regardless of the scientific judgments by experts who will develop and consider these applications, a hostile public or one unconvinced of the value of these developments will give the biotechnology industry a difficult time in the marketplace. Several proposed field tests have already been the targets of protest in some communities, although other proposed field tests have met with little or no local opposition.

European and Japanese Regulation

In addition to action in the United States, several European nations have begun to assess the need for regulatory review of genetically engineered organisms in the environment. Both the European Economic Community (EEC) and several member nations have considered regulatory issues over the last few years.

The EEC established a biotechnology steering committee in February 1984 to coordinate biotechnology policies. In 1986, a meeting was held by member-state officials to discuss the regulation of releases of genetically engineered organisms in the environment (35). In November 1986, a commission report highlighted the need for collective action, and announced that proposals would be developed for Community action on "a) levels of physical and biological containment, accident control, and waste management in industrial applications, and, b) authorization of planned release of genetically engineered organisms in the environment" (8). This initiative occurred as several member states were taking steps to regulate biotechnology:

- **Denmark** enacted legislation in 1986 preventing the deliberate release of any organism that is the product of recombinant DNA technology as well as any organism resulting from gene deletion or cell hybridization (23). The Danish law forbids such experiments unless approval is obtained from the environment minister.
- **France** in 1987, established a 15-member panel of scientists, under the jurisdiction of the Ministry of Agriculture, to review proposed deliberate release experiments on a

case-by-case basis and to consider the need for future regulation. Notification of any intent to use recombinant DNA technology must be made to the Ministry of Research and Higher Education (23). Field tests involving the nitrogen-fixing bacteria *Rhizobium* began in March 1987.

- **The Netherlands** established an advisory committee on recombinant DNA activities to regulate such research.
- **The United Kingdom** developed voluntary guidelines published in April 1986 that encourage any person planning a deliberate release of an engineered organism to contact the Health and Safety Executive (9). Proposed regulations would require scientists to notify the Executive of any general intention to conduct experiments involving genetic manipulation as well as individual notification for certain high risk experiments (27).
- **Sweden** established a special commission in 1984 to study whether tighter regulations were needed for recombinant DNA research. The advisory committee recommended that existing occupational health and environmental protection oversight is adequate, and that stricter regulations are not needed.
- **The Federal Republic of Germany** classifies experiments in four categories; releases of organisms into the environment fall into the prohibited category, although researchers may apply for an exception. A parliamentary commission was formed in 1984 to study the potential scientific, social, and legal implications of gene technology. The report recommended a 5-year moratorium on environmental releases of genetically altered viruses (except for those used as vaccines in human and veterinary medicine) and of micro-organisms into which genetically foreign genes have been inserted (8).
- **Japan** regulates biotechnology through several ministries. Pharmaceutical production has developed under guidelines developed by the Ministry of Health and Welfare. Regulation of agricultural biotechnology is expected to become more important as the number of permits for research and production increase (21).

SUMMARY AND CONCLUSIONS

A recent poll indicates that a large majority of Americans (82 percent) approve of small-scale experimental tests of genetically engineered organisms for environmental applications. Most people approve of such applications for a variety of purposes, and a majority appear willing to accept relatively high levels of risk to the environment in exchange for the potential benefits that might be derived from environmental applications of genetically engineered organisms.

The experiences of local communities illustrate the varying degrees of local support for field tests of genetically altered plants, animals, and micro-organisms. In several instances, local opposition thwarted or delayed proposed field tests. In other communities opposition was minimal. Opponents of proposed field tests have relied on State environmental laws, local laws (e.g., zoning laws, county ordinances), political pressure (e.g., petition drives, public meetings), and even physical sabotage of test sites to achieve their objectives. Where opposition has been minimal, companies and individual researchers have generally informed governmental and citizens' groups about their scientific goals and objectives, the degree of regulatory review of the experiment, safety considerations, and the economic impact of such experiments on the local economy.

Factors specific to individual cases may affect the degree of public support or opposition to a proposed field test. Genetically altered micro-organisms, for example, have elicited more public concern than proposed field tests of plants. The extent of local support or opposition may also depend on the degree to which a proposed field test is perceived as a first (e.g., the first release of a micro-organism, first application under TSCA, first release in a particular State).

The development of recombinant DNA techniques during the 1970s led to self-regulation by scientists and, later, regulation by the Federal Government. The Coordinated Framework for Regulation of Biotechnology, published by the White House Office of Science and Technology in 1986, describes a comprehensive Federal regulatory policy to ensure the safety of biotechnology research and products. Several challenges face regulators as they adjust to the new Framework: defining key terms; balancing risks and benefits of the new technology; maintaining a favorable economic climate for research and product development; assuring that regulation of one type of product does not hinder development of other products; determining jurisdiction when regulatory oversight or review is to be performed by more than one Federal agency; balancing technology promotion and regulation; establishing consistent penalties for violators; resolving potential challenges to the legality, applicability, and scope of current statutes; balancing technology promotion and regulation; establishing consistent penalties for violatons; resolving potential jurisdictional conflicts between Federal, State, and local governments; and assessing public opinion.

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Chapter 4

Genetic Considerations

"We should assume that on an absolute scale the transfer of genetically active DNA is a very common event, and that it generally occurs with no harmful consequence to host cells already well adapted to their own genetic messages."

James D. Watson

Bulletin of the Atomic Scientists 33:12-13, 1977

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Chapter 4

Genetic Considerations

The safety questions raised by the planned introduction of genetically engineered organisms are not unique to such organisms. Yet the introduction of these organisms raises some safety questions that are quite different from the questions of physical containment on which previous discussions of the safety of recombinant DNA research have focused. And though the intimate interplay between the genes of an organism and the environmental parameters that govern the way the genes are expressed makes most separations of genetic and environmental factors difficult, such divisions make the issues easier to examine. This chapter focuses primarily on genetic issues, particularly as they relate to the potential for movement of engineered genetic material beyond the intended host. Most genetic factors are either important primarily as they relate to this potential, or are more clearly relevant in an ecological context, and are thus discussed in chapter 5.

The migration of genetic material, or **horizontal transfer, is the passage of genetic material from one organism to another through a mechanism not involving specialized reproductive cells** (i.e., nonsexual gene transfer). In bacteria, it is the transmission of genetic material from one contemporaneous bacterial cell to another, by any of several means. OTA assumes that genetic material introduced into a host in which it does not naturally occur has some finite probability of migrating to a nontarget organism. What is that probability? How can the movement of the genetic material be observed? What are the potential consequences of horizontal gene transfer? And what steps can be taken that would limit the frequency or mitigate the potentially adverse consequences of horizontal transfer?

An OTA workshop, convened in collaboration with the National Science Foundation, examined these and other questions surrounding genetic issues in the planned introduction of genetically altered organisms. This chapter summarizes some of the factors that could influence the frequency of horizontal gene transfer after the planned introduction of recombinant organisms and examines technologies designed to affect horizontal transfer. Most of this chapter is relevant primarily to microbes and much less relevant to higher organisms such as plants or animals, unless specified.

WHAT IS KNOWN ABOUT HORIZONTAL TRANSFER OF GENES?

Genetic material probably does not often move across large evolutionary distances, between organisms only remotely related. When gene transfer does occur, it appears to take place via a limited number of mechanisms. Different types of organisms, such as bacteria and plants, share some mechanisms for genetic transfer, while other mechanisms are specific or unique to particular organisms (2,14,15).

The different types and mechanical details of gene transfer have been discovered and examined primarily in controlled laboratory situations. Gene transfer between bacteria under laboratory conditions has been widely described, and there is evidence that transfer in natural ecosystems (e.g., in soil, in aquatic systems, or on plant surfaces) does occur (12). Horizontal transfer between plants has not been well studied, and no conclusive data exist to indicate that it occurs in nature. But nonsexual genetic exchange involving the insertion of bacterial material into plants is well documented. Gene transfer has been investigated and described in invertebrate systems, particularly insects. Evidence from the evolutionary record suggests that some rare horizontal transmission of genetic material has occurred between mammalian species. Although there is no firm evidence that genes are passing back and forth between diverse groups of organisms, there are instances that warrant further investigation.

Although several specific systems of genetic transfer have been studied, they probably represent only a subset of what actually occurs in nature. In fact, since these systems have been selected for intensive research because of their ease of handling, the likelihood of producing rapid results, and their accessibility to existing research methodologies, their role in horizontal gene transfer in nature may be overestimated. Indeed, many barriers to gene transfer exist in natural systems. Thus, many questions remain to be answered about gene transfer outside the laboratory, including:

- How extensively do the gene transfer mechanisms observed in the laboratory operate in nature?
- What are the genetic and environmental conditions under which novel information could be incorporated into a foreign genome and subsequently expressed?
- Do populations of organisms limit incursion of new genetic material, and if they do, by what means?

WHAT GENETIC CONSIDERATIONS MUST BE EVALUATED IN PLANNED INTRODUCTIONS?

The planned introduction of a genetically engineered organism raises three issues of immediate concern. First, if gene transfer does occur, will the new genetic information be maintained and expressed? Second, what is the potential extent of horizontal transfer of manipulated genetic material? And third, if the modified organism, or the inserted DNA it contains, moves beyond the point of introduction, how will it affect the surrounding populations or communities of plants, animals, and microbes? This issue, regarding ecological considerations, is discussed in chapter 5. The first two questions, of horizontal transfer and expression, are considered here.

Some commentators have maintained that if the gene in question will not move to other organisms then there is no need to worry about potential consequences of its introduction. Others maintain that if the modified organism or gene of interest would not cause problems even if it moved, then the exercise of estimating transfer probability is unnecessary. Both issues should be addressed in assessing potential consequences of a proposed introduction experiment (see ch. 6), although in some cases not enough is known about the life histories of organisms that could be involved to make such hazard estimates possible. But a very low probability of transfer multiplied by a moderate probability of expression and resultant hazard if transfer occurs is a different situation than if both probabilities are very low. By the same token, a significant probability of benefit could offset all or part of any potential risk.

Many factors influence the magnitude, frequency, stability, and effects of horizontal gene transfer to nontarget organisms. Identifying these factors is necessary if scientists, corporate administrators, and government regulators are to evaluate environmental applications of genetically engineered organisms. Table 4-1 lists the minimum factors that must be considered, which are discussed in the next section.

Predicting the Potential Effects of Horizontal Transfer

Can generic rules be discovered that would help distinguish a condition of low probability of hori-

Table 4-1.—Some Genetic Factors To Evaluate in Planned Introductions

Possible method of manipulation	Factor
Organism choice or design	Gene Vector Construct Host organism Recipient organism
Population manipulation	Survival of released organisms Population density of host
Other means	Presence of potential nontarget organisms Density of potential nontarget organisms Selection pressure

SOURCE: Office of Technology Assessment, 1988 zontal gene transfer from one of high probability? What types of accessible information yield likely estimates of the magnitudes of horizontal gene transfer? These questions are difficult to answer with precision.

An analysis of the **magnitude** of horizontal gene transfer must include at least two components:

- an estimate of the **frequency** of gene transfer from introduced to nontarget organisms, and
- an estimate of the **genetic distance** between the original organism and the nontarget species.

The consideration of both **intrinsic** and **extrinsic** factors can help assess the likely extent of horizontal gene transfer. Intrinsic factors, which are elements of molecular biology, include:

- the **host** organism used in the application (e.g., plant or micro-organism);
- the **gene** being manipulated (e.g., gene conferring pesticide resistance or ice-nucleating activity);
- the **vector** introducing the gene into the host organism (e.g., a plasmid or virus); and
- the **construct,** or final configuration of the new genetic material within the host organism, which will govern expression and stability of the gene product.

Extrinsic factors, which are elements of ecology, include:

- the **survival** of the released host organism,
- the presence of **potential nontarget recipients** of the gene and the evolutionary relationship between the host and potential nontarget organisms,
- the **population densities** of the engineered host and the potential nontarget recipients in the environment, and
- the **selection pressures** to maintain the new gene in either population.

By influencing the magnitude of horizontal gene transfer, these extrinsic factors become integral to any examination of genetic considerations of environmental release.

Intrinsic Factors

The impact of intrinsic factors on horizontal gene transfer cannot be measured by simple descriptive information about the host, gene, vector, and construct. A number of principles help explain and predict the behavior of genes. Before estimating the frequency of horizontal transfer in a system, the natural histories of each component must be understood. The information should include, but not be limited to, how the gene is expressed in different environments, both genetic and ecological; the behavior of the vector in different hosts; and the different life stages, if any, of the host.

Host

It is important that the micro-organism, virus, fungus, plant, or animal used as the host be well understood, and its life cycle well studied. Perhaps most important, the mechanism(s) by which the organism transfers genetic material in the laboratory should be identified. For instance, one class of bacteria (called gram-negative) usually uses plasmids or phages to facilitate genetic exchange. Another class (gram-positive) uses the direct exchange of DNA segments as an important mechanism of gene transfer.

Although the bacterium *Escherichia coli* is well understood, less is known about genetic exchange by bacteria outside the laboratory, especially in soil. In some instances, nondebilitated bacteria are being developed for planned introductions despite the paucity of information on host survival, genetics, and population structure. Some of these organisms may survive and function for long periods in their new environment (13). **Without a well-developed natural history of host organisms, it is impossible to evaluate the genetic and ecological implications of a planned introduction**. Substantial experience with past microbial introductions indicates, however, that even when introduced bacteria survive, they do not come to dominate the host community.

The presence of cryptic genetic material (e.g., cryptic plasmids) is a host characteristic that merits special consideration. Cryptic genetic ma-

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"There is no problem. Any damage caused by the nuclear accident can easily be remedied by genetic engineering."

terial appears to have no assigned function and is often assumed to be inactive. But its function may depend on environmentally induced stimuli, a condition that could make it appear nonfunctional in the laboratory. A gene on a cryptic plasmid, for example, might be expressed only under starvation conditions, a common condition of bacteria in nature but not in the laboratory.

One species of *Yersinia* provides an example of differential gene expression. This bacterium carries a plasmid that produces four or five important gene products only when the organism is growing within its natural environment. Another example can be seen in the difference between *E. coli* in the test tube and in its natural habitat, the gut. Some *E. coli* plasmids code for adhesion factors that allow the bacterium to colonize the gut. These plasmids are expressed only when the bacterium is in the gut. Thus, genetic material that appears to be cryptic in the laboratory could have important functions in nature. So it is theoretically possible that a seemingly dormant piece of genetic material could provide the mechanism for an engineered gene to be transferred from the host to a nontarget organism. This possibility makes it important that the life history of the organism be understood before declaring genetic material cryptic or before using as hosts strains of bacteria carrying cryptic plasmids, at least in situations where it is imperative to avoid the possibility of gene transfer.

On the other hand, the natural histories of many host organisms are well known. One such host is *Pseudomonas fluorescens*. Scientists have proposed using this bacterium, altered to carry the delta-endotoxin gene from *Bacillus thuringiensis*, to protect corn roots from the black cutworm. The toxin kills the cutworm that feeds on the corn rootlets. The *P. fluorescens* host was chosen because it has been well studied and is easily identified. Since the toxin gene has been inserted into a new host organism, however, the probability and frequency of the gene being transferred out of the engineered *Pseudomonas* is a valid question. Do the mechanisms that *B. thuringiensis* uses to exchange genetic material differ from those of *P. fluorescens?* Is the frequency of gene transfer different between the two, or do they typically exchange genes with the same or different species? Do restriction enzyme systems in potential nontarget recipients reduce the probability of transfer of intact DNA? Host-related questions such as these may be important in assessing genetic considerations of planned introductions.

Gene

Ideally, planned introduction experiments would involve genes that have a well-understood natural history as well as host organisms that have been studied thoroughly. An extensive natural history would help determine whether potential new interactions between the gene and the environment could result from the gene's presence in a new host microenvironment. How specific should or could applications for approval of planned introduction field tests be about gene-environment interactions? Can any novel expression of phenotype occur? Unfortunately, these questions are impossible to answer, because they require scientists to predict and quantify the occurrence of rare and idiosyncratic events. Only gradually increasing experience will start to provide answers.

Again, the toxin gene of *B. thuringiensis* is a good example of a gene from an organism with a well-characterized natural history (1). The naturally occurring bacterium and derived materials have been used for decades. They are now available in over 410 different products, in 13 formulations (e.g., powder, pellet, or solution) to apply the toxin in garden, agricultural, and forestry settings (6). In many areas containers of *B. thuringiensis* spores can be purchased at garden stores. Tons of the bacterium have been applied to agricultural and forestry lands. Despite intensive searching, scientists have unearthed no evidence to date that either the endotoxin gene has escaped from the *Bacillus* and been expressed in other microbes, or that the toxin from this strain (var. *kurstaki*) has any effect on organisms other than Lepidoptera and closely related insects.

Vector

Vectors are the means by which genetic material is shuttled between organisms. Just as it is necessary to have a well-characterized host and gene, it is important to use a vector with well understood characteristics. Important factors include the vector's ability independently to initiate or sustain horizontal gene transfer, its need for outside help to move information, and its degree of mobility or the extent of its host range.

Construct

An important factor affecting the probability that an inserted gene might move from an altered host to a nontarget organism is the final configuration of the new gene in the host—i.e., the DNA structure at the site of gene insertion. For instance, inserting a gene into a chromosome minimizes subsequent gene movement, especially compared with inserting it into a plasmid. The source of the regulatory sequences controlling expression of the inserted material is also important and plays a major role in limiting the field of potential nontarget recipients.

Genetically engineered "ice-minus" bacteria also illustrate the importance of construct to the likelihood of horizontal gene transfer. This bacterium is created by removing a gene found naturally in *Pseudomonas*, *Erwinia*, and other bacteria, a construction that decreases the probability of horizontal gene transfer.

The transfer of a deletion—in this case, essentially a missing gene—to a nontarget organism cannot impart a new capability to the recipient in the same way that acquiring a novel structural gene can, as in the case of the *B. thuringiensis* toxin gene. So even if the altered genetic material is transferred beyond the host, it cannot add to the nontarget recipients the ability to produce a new gene product. Deletions can, however, alter the relationships of the host species to other organisms with which it interacts, a change that could be important under some circumstances.

Extrinsic Factors

The extrinsic factors that strongly influence the likelihood and magnitude of horizontal gene transfer are an integral part of the environment into which the engineered organism is introduced. The expression of the trait, the intended environment, and other environments that the engineered organisms could encounter must be analyzed for their possible impact.

Survival

A key determinant of potential horizontal transfer is the ability of the introduced organism to establish and reproduce itself in its new habitat, and to stably express the engineered trait. Unfortunately, little information exists on the potential survival, establishment, growth, and subsequent genetic transfer ability of engineered organisms placed as competitors to indigenous organisms in a natural environment (13), though most evidence suggests survival is most likely to be diminished. Laboratory conditions are artificial and differ significantly from those encountered by organisms in their native habitat. For example, the mean generation time for many bacteria in soil is about six months (although this figure varies widely for different soil organisms and with the season), compared to one hour or less under laboratory conditions. The time of year and the local qualities of individual introduction sites could also affect survival significantly.

In one experiment, naturally occurring *P. fluorescens* were isolated from corn roots and given genetic markers to allow them to be detected at a later time. The organisms were then reinoculated onto the corn roots at a moderate density. During the following growing season it was difficult and in some cases impossible to reisolate the marked organisms. Thus, a soil system (and perhaps other natural habitats) might not contain enough nutrients to allow measurable survival of laboratory-adapted micro-organisms. Certainly for genetically engineered micro-organisms (and perhaps other organisms), the problem will likely be less one of persistence and gene transfer than of survival to perform the job for which they were designed.

Potential Nontarget Recipients

For horizontal gene transfer to take place, a compatible recipient must be available. The most likely recipient is an organism genetically similar to the engineered host. The probability of transfer generally declines as evolutionary relatedness decreases. Restriction enzyme systems that degrade evolutionarily unrelated "foreign" DNA are common among bacteria.

Information about the natural history of potential nontarget organisms in the environment, however, is scarce—less than for laboratory-engineered organisms. In the case of bacteria for agricultural applications, potential microbial recipients in soil are of interest. Yet only about 10 percent of the microbial species in soil can even be cultured in the laboratory.

Horizontal transfer of genetic material between higher organisms is less likely than that between simpler ones. However, gene transfer via sexual recombination among these organisms could be an important problem. In particular, genetic movement via natural sexual transfer from crop plants (e.g., engineered to be herbicide resistant) to related weedy species could occur. Such problems are neither new nor unique to engineered plants, however (see ch. 5), and the processes involved are understood.

Density

Important factors affecting the magnitude of horizontal gene transfer are the absolute densities of the introduced and recipient organisms. According to laboratory research with bacterial systems, the rate of transmission seems to be proportional to the product of the densities of the donors and recipients.

In the case of micro-organisms, it appears that the numbers of naturally occurring nontarget recipients in the environment (e.g., in soil or water) are low—considerably lower than the concentrations necessary for efficient gene transfer in the laboratory. For instance, among organisms that are well studied, the number of naturally occurring organisms in fertile soil is normally at least an order of magnitude lower than concentrations of bacteria necessary for horizontal transfer in the laboratory.

Density can also be affected by the method used to introduce an engineered organism. Additionally, the timing of the planned introduction can affect the density of both the engineered organism and potential nontarget recipients. But keeping introduction densities low to avoid gene transfer may not be consistent with an effective introduction, since high initial density and survival may be required for efficacy.

Selection Pressure

The probability that new genetic material will persist, be expressed, and increase in frequency in nontarget populations if transmitted is at least as important as the probability of horizontal gene transfer itself. Selection pressure is the major determinant. A low probability of positive selection —i.e., little likelihood of the persistence of the new material—is usually the desirable outcome.

Selection pressure is determined by a combination of factors, including the trait encoded by the engineered gene, the potential recipients, and the value of the trait in the introduction environment. Because environmental conditions are generally harsh and stringent (e.g., inadequate nutrients for growth, and suboptimum temperature conditions), selection pressure is crucial. Under usual conditions (i.e., the gene product does not confer a selective advantage), even a moderate amount of new DNA assimilated by an indigenous soil or water micro-organism may impose enough of an energy drain that the organism will be selected against in competition with others that do not carry additional DNA. However, **different introductions will vary with respect to the important selection pressures, and they must be evaluated separately.**

Even a low horizontal transfer rate can establish the trait in a new species if assisted by strong selection pressures. Although some individuals point to the rapid spread of antibiotic resistance in gonococcus as an example of the widespread problems that can occur when genes are horizontally transferred, it is important to realize that intense selection pressure exerted by indiscriminate and subtherapeutic antibiotic use, especially in foreign countries, was probably the overwhelming cause of this phenomenon. The development of penicillin resistance by gonococcus illustrates the power of selection pressure to overcome such seeming obstacles as low rate of transfer.

TECHNOLOGIES TO MONITOR HORIZONTAL GENE TRANSFER

Beyond the intrinsic and extrinsic factors that could affect the magnitude of horizontal gene transfer in environmental applications, it is important to examine risk management methods that could be used to monitor both the dispersal of altered organisms and the movement of genetic material. Because proposals to introduce genetically engineered organisms are still new, detection or tracking methods are not highly developed. Experience (e.g., with past introductions of rhizobial or plant pathogenic bacteria) suggests that although such tracking methods will be needed in the future, their current level of development presents more inconvenience than danger.

An important distinction in monitoring is the difference between tracking the organism and tracking the gene or construct of interest that the organism carries. Improved methods to do both have been identified as one of the major unmet research needs in this area (see ch. 6). Some tracking technologies are now available.

Selective Screening Methods

One tracking method is based on the ability of researchers to mark a host organism's chromosome with genetic characteristics, such as antibiotic resistance genes or nutritional markers, that will confer an advantage to the organism when placed under specific conditions in the laboratory. These selective methods, principally used with micro-organisms, increase the probability that an investigator can isolate the test organism from the environment if it has persisted.

While useful in the laboratory, markers that could confer an unintended selective advantage in the environment, either to the host organism or nontarget recipients, should be avoided if possible, and carefully evaluated when used. One study concluded that "it is essential to choose antibiotics which are not in use in humans or animals, since resistance to clinically useful antibiotics is a major public health problem" (10). The example of penicillin-resistant gonococcus, cited earlier, underscores this point. But even the large-scale introduction into the environment of genes for resistance to nontherapeutic antibiotics should be carefully evaluated. Some resistance genes could mutate to counter whole families of related antibiotics. The kanamycin resistance gene, for example, could acquire the ability to neutralize newer antibiotics derived from streptomycin.

Some individuals, however, argue that the introduction of resistance genes is unlikely to cause problems, especially in land applications. The argument is based on two considerations, both involving micro-organisms. First, many resistance genes are already present in soil micro-organisms. In fact, this background of resistance could hinder tracking efforts, a problem that will almost certainly require the use of multiple selective markers. Second, studies of root ecology have long involved the use of antibiotic resistance with no apparent adverse effects.

Technologies using selective methods to track the genetically engineered gene itself are under development and promising approaches have been designed. The antibiotic resistance strategy puts a resistance marker near the gene of interest. The antibiotic could be used to recover any cells containing the resistance gene. In most cases, the gene of interest—the inserted gene—would travel with the antibiotic resistance marker. So obtaining and quantifying cells that are antibiotic-resistant would allow the measuring of horizontal gene transfer to nontarget species. Another approach avoids the use of antibiotics and employs a metabolic marker such as *lac*, which brings the capacity for metabolism of lactose, as a convenient, innocuous, but effective tracer gene. The *lac* gene is inserted close to the gene of interest so that it may serve as a linked marker.

Biochemical Screening Methods

A different approach to the tracking problem employs gene probes constructed through recombinant DNA technology. A segment of DNA that is complementary to the gene, or DNA sequence, of interest serves as the probe. The segment is labeled with radioactivity, a specific dye, or other tag that can be easily detected in the laboratory. A sensitive method, this gene probe technique may identify both host and nontarget recipients of labeled material. Similarly precise identification is also possible with antibody probe analyses derived from monoclonal antibody technology.

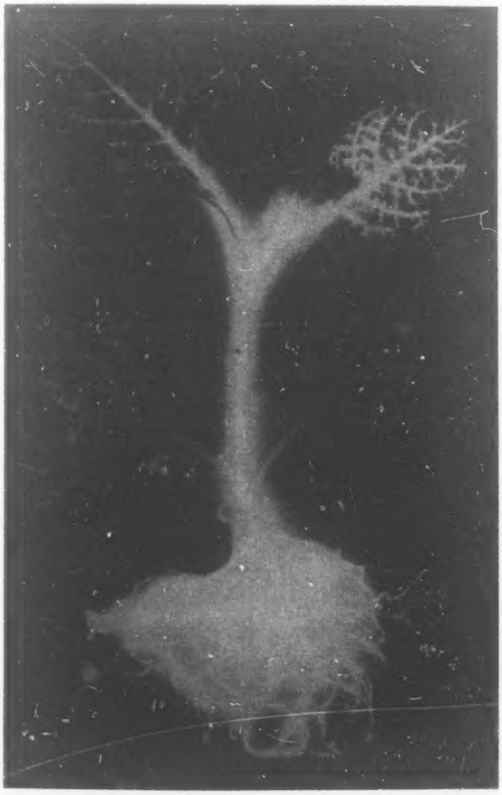
To apply these methods to bacteria or viruses, the organisms would be isolated from the environment and their DNA extracted and tested with the specific gene or antibody probe. With plants, leaves and other parts would be obtained and their DNA extracted and tested with the probe in the laboratory. By binding to an organism's DNA, the probe reveals that the organism carries the gene of interest. Such probes allow the detection of an inserted gene regardless of its position in the host's or recipient's genome. In contrast, the selective screening method just discussed becomes useless if the inserted gene becomes separated from a closely linked selection marker.

One disadvantage of the gene probe is that it provides no means of discriminating between samples that should be tested and those that should not. Everything that grows out of a sample (e.g., micro-organisms from the soil or a river, or plants from a wide area) must be screened. Furthermore, as mentioned earlier, many native micro-organisms cannot be cultured in the laboratory. Thus, these probe methods of tracking will probably be more useful to detect and monitor the presence of host organisms than to quantify horizontal gene transfer.

Research projects based on the gene probe concept are now being funded by the Environmental Protection Agency. The general applicability of biochemical monitoring appears promising. In the case of soil micro-organisms, extracted DNA must be purified sufficiently to meet laboratory conditions for the test to be accurate. In water applications, enormous volumes of water often must be processed to obtain test samples.

A related biochemical tracking method uses the luciferase gene cloned from fireflies. This gene codes for a light-emitting protein, luciferin, and has been inserted into plants and cultured plant cells that now "glow in the dark." Although the presence of the luciferase gene can be detected through the probe methods just described, it can also be detected easily through image intensifying video equipment or by contact exposure to photographic or x-ray film (see figure 4-1) (11).

Figure 4-1.—Luminescence From a Tobacco Plant Containing the Firefly Luciferase Gene



SOURCE: D.W. Ow, K.V. Wood, M. DeLuca, J.R. deWet, D.R. Helinski, and S.H. Howell, *Science* 234:856-859, 1986.

Using the firefly gene as a marker could be a fast, easy, and useful method to track genetically engineered organisms. It is very energy-intensive for the host organism, however, and its utility is therefore likely to be limited.

TECHNOLOGIES TO PREVENT OR REDUCE HORIZONTAL GENE TRANSFER

In addition to the technologies being developed to track host organisms and genes, methods are being developed that manipulate intrinsic factors so that introduced organisms have:

- a lower probability of persisting after introduction,
- a lower probability of transferring genetic material, or
- both.

Methods to prevent or reduce horizontal gene transfer are straightforward. The genetics and biochemistry of conjugal transfer have been well studied. By mutagenesis or genetic engineering, the capability for mobilizing DNA for transfer, and the genes specifying the necessary cellular apparatus for the transfer, can be removed from a plasmid. The plasmid can be further debilitated so that it is only poorly mobilized in the presence of another, potent plasmid. Such disabled plasmids are not capable of detectable horizontal gene transfer, and these disabled plasmids are commonly used at present in genetic engineering in bacteria.

Although the application of disabling methods may be an important component of planned introductions of genetically engineered organisms, the specific type of organism involved must be considered. Even the most active plant vectors, for example, probably have a lower likelihood of horizontal transfer than the least active bacterial vectors. Furthermore, it appears that genetically altered organisms that derive their utility by being deprived of a trait (i.e., a deletion) are less likely to be able to produce problems via gene transfer, although this might not always be the case (3,9).

Experience in working with recombinant DNA organisms in the laboratory provides some examples of success in restricting unintended gene movement by disarmament measures. These approaches, specifically the use of crippled bacteria and plasmids, have served as the starting point in developing ways to prevent or reduce horizontal gene transfer.

Debilitated Host Organisms

The degree to which a host organism should be debilitated will, again, depend on its intended application. In the case of a bacterium that will be used to degrade a toxic chemical, it might be prudent to use a self-destructing organism, since the bacterium only need persist as long as the pollutant is present. On the other hand, if the bacterium were designed to protect a plant from an insect pest, the organism's persistence in the soil might be desirable (but see ch. 5).

One of the earliest attempts to construct a debilitated organism arose from the original questions surrounding the first uses of recombinant DNA technology. Although several studies had established that the organism initially used in recombinant DNA experiments (*E. coli K-12*) did not colonize the human intestinal tract (even after ingestion of billions of organisms by volunteers), a severely crippled strain of *E. coli K-12*, designated χ^{1776}, was developed. This further debilitated derivative was, however, quite difficult to work with even in the laboratory. With experience, the original K-12 strain has proved to be an extremely successful and effective form of biological containment. The use of debilitated organisms in field tests, however, might compromise the value of the test, and therefore may not be a generally desirable approach.

Another approach sometimes suggested for reducing the chance of gene movement is to engineer restriction systems, common defense systems in naturally occurring bacteria, into a host bacterium. Restriction enzymes degrade unprotected DNA, so that foreign DNA from a donor is unable to infect the host. They are common enough in natural populations of bacteria that they can be expected to play a significant role in deterring the transfer of genes from introduced engineered organisms. In addition, such naturally occurring restriction systems might be adapted to help inhibit transfer of the inserted gene out of the engineered host, or otherwise limit its function or persistence. At present, such systems are not well developed, but they hold substantial promise.

Disarmed or Nonmobile Vectors

In addition to engineering crippled host organisms, more stable vectors— those that would have little probability of facilitating genetic movement —are being developed. In particular, efforts are focused on obtaining microbial and viral vectors that are "escape-proof."

The concept of a debilitated host micro-organism (e.g., *E. coli K-12*) was also applied to the development of a vector for that system. Plasmid pBR322 was isolated and has been used as a vector for transferring engineered genes in the laboratory. The plasmid is incapable of self-initiated transfer and is also poorly mobilizable. It is therefore considered safe; it has a low probability of being transferred to bacteria indigenous to natural habitats, including the human gastrointestinal tract. Similarly crippled vectors have been developed for use in insects and for mammalian genetic engineering.

Another precaution suggested by the gonococcal resistance example (see box A in ch. 1) is to reduce or eliminate the use of mobile transfer elements in engineered organisms. Since some vectors are clearly more mobile than others, using disarmed versions of these vectors, or avoiding their use entirely, would reduce the probability of horizontal gene transfer.

A disarmed vector (a transposable element) was the approach used in the insertion of the toxin gene into *P. fluorescens*. This technique appears to be successful, and the application to field test the organism is pending. Experiments show that it is unlikely that the nonmobile transposon will be excised (7,8).

Finally, an EPA research group is attempting to construct a "suicide" bacterium designed to persist in the environment only as long as it is needed. The organism is a bacterium that contains a vector (in this case a plasmid) that will self-destruct in the absence of the toxic substance it has been designed to clean up. A better name for this technique might be "suicide plasmid," since the main purpose is to destroy the vector DNA before it transfers to another host. Other groups are also working on different means to similar ends (4).

However, the demonstrated ability of free DNA to sometimes maintain its integrity in soil or water creates a potential problem. If the cell were killed before the plasmid had self-destructed, and the plasmid with its inserted gene remained intact, it is possible that the plasmid could enter another cell.

SUMMARY AND CONCLUSIONS

The planned introduction of genetically engineered organisms (chiefly bacteria and plants) stands as the next research step in the anticipated biotechnological revolution. Although genetically altered organisms isolated through traditional genetic methods have been widely used in the environment for decades, the prospect of widespread application of genetically engineered organisms has heightened the concern of some that increased problems may arise. "The implication for R-DNA-engineered organisms is that large-scale or sustained applications might have consequences different from small-scale or single applications . . . the cumulative probability of undesirable effects resulting from repeated applications or frequent introductions must be considered" (5). Another potentially important question about the planned introductions concerns the possibility that an introduced organism might transmit its novel genetic material to nontarget hosts, resulting in unintended and possibly adverse consequences.

Most of what is known about horizontal gene movement has been discovered in laboratory studies. Little information is available on how the phenomenon occurs in nature. There appear to be a limited number of gene transfer mechanisms; research has revealed that different types of organisms share some mechanisms for genetic transfer. Genetic material is not generally thought to transfer across large evolutionary distances, however, and there are numerous impediments to gene transfer, even between closely related species.

For regulators to assess the potential genetic impact of an engineered application, several factors must be evaluated for their effect on horizontal gene transfer: intrinsic factors, such as the host organism, gene, vector, and construct, that are elements of the molecular techniques used to create the engineered organism; and various extrinsic factors that are elements of ecology, including survival, potential nontarget recipients, density, and selection pressure. Several methods can now be used not only to monitor survival of introduced organisms and genes, but also to reduce or prevent horizontal gene transfer.

Generic factors that can serve as a framework for regulation can be described. Technological advances exist or are being developed to protect the public and environment from any unintended consequences of introducing altered organisms. Some introductions merit closer scrutiny than others, and **OTA finds that evaluation of proposed applications to introduce into the environment genetically engineered organisms which are believed to carry some element of risk should proceed on an adaptable, case-by-case basis**, at least until knowledge has been accumulated to make more general reviews feasible. With an adaptable, case-by-case review of such planned introductions, not only the current spectrum of genetically engineered organisms, but kinds as yet unanticipated should be able to be tested in the environment without unreasonable risks. The current range of genetically engineered organisms seems to have a low probability of creating problems, particularly via the horizontal transfer of genetic information to nontarget recipients. However, this does not mean there are no risks at all. Careful regulation and enforcement can guard against potential environmental or public health problems and protect the biotechnology industry from the backlash and loss of credibility and confidence that a severe problem could precipitate.

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Chapter 5

Ecological Considerations

"Ecology could easily provide an endless catalogue of reasons for never interfering with the biosphere. What we should be doing is to insist on adequate ecological input into the screening of release proposals, together with a determination to extend that knowledge by case law as experience accumulates."

Bernard Dixon
Bio/Technology 4:481
June 1986

"Experts, except for those operating under the banner of ecology, generally oppose the introduction of ecological questions into their problems."

Garret Hardin
Filters Against Folly, p. 56
Penguin, New York, 1985

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Chapter 5

Ecological Considerations

In 1987, a report by the National Academy of Sciences stated "an urgent need for the scientific community to provide guidance to both investigators and regulators in evaluating planned introductions of modified organisms from an ecological perspective" (39). This chapter incorporates such a perspective and focuses on a number of ecological issues relevant to the planned introduction of genetically engineered organisms into the environment.

The major concern associated with planned introductions stems from the potential for problematic ecological effects that are unintended or unforeseen. With appropriate regulatory oversight, such effects are unlikely to follow from any planned introductions in the near future. If they occurred, however, they might be felt at any level—from the local population, through the community and ecosystem, to fundamental ecological processes—although the probability of such disruptions decreases as their severity increases. A number of ecological questions are explored in this chapter, including the relevance of experience with introduced exotic organisms, the types of disruptions to natural populations that could take place, and the potential for effects on ecosystem processes of energy flow and nutrient cycling. Review of the data bearing on these questions suggests that **there is some reason to be cautious, but no cause for alarm at the prospects of planned introductions of genetically engineered organisms into the environment**.

GENETICALLY ENGINEERED ORGANISMS AND EXOTIC SPECIES

Studies of biogeography—the distributions of plants and animals—demonstrate the presence in most modern environments of nonnative or exotic organisms that evolved elsewhere. The number of these exotic species (microbes, plants, and animals) is large, as a result both of natural processes of dispersal and deliberate and accidental distribution aided by humans. No good data indicate what proportion of introduced organisms has created ecological problems, although it is generally agreed to be small. Some scientists believe that experience with exotic species provides an example of what might be expected from genetically engineered organisms in planned introductions, and that "the history of introductions is a history of disasters that should not be forgotten lest it be repeated" (23). Others argue that the best guide is the experience with past introductions in agriculture of organisms produced by hybridization and crossbreeding—a history of far more positive results, and one in which negative consequences have been met with a variety of existing control and mitigation strategies. They point out that all major crops in the United States are exotic species introduced from other regions of the world, and that it is often important to separate the effects of planned introductions from those of accidental introductions. Although no existing database provides all the information needed to answer questions in this area, there are several different sources of relevant data.

Introductions of Exotic Species

A wide variety of reports have been published on invasion or colonization by exotic species (16, 17,23,37,57). Some of the most quantitative data available deal with insect introductions. One study reveals an exponential increase in the number of insect species introduced into the area of the 48 contiguous States from 1640 to 1977 (table 5-1) (46). No data are available on the number of potential insect introductions that failed to become established, but it is assumed to have been far larger than the number that succeeded (46, 56). Of the 1,379 species noted as having been successfully introduced, some economic importance is assigned to 1,089 of them (79 percent). This percentage is almost certain to be an overestimate since insects with no economic impact draw scant attention and may inadvertently be excluded from the total of successful introductions. The economic significance of those introductions that were noted is further broken down in table 5-1.

One-fifth of the introductions studied had some beneficial impact; another fifth had no significant impact. Two-fifths (41 percent) of the successful introductions were of insects that turned out to be minor pests. About one in five introductions had severe, negative consequences (17 percent). The majority of these negative effects were unexpected, because no such impacts were known in the introduced species home ranges; the new environment apparently lacked constraints that operated in the original areas. If more had been known about the life histories of these insects, the kind of effects that followed their introduction to the new environment might have been anticipated.

One review of the history of plant and animal introductions (57) concluded that in nearly 80 percent of the cases examined (678 of 854), introduced species had "no effect whatsoever on species in the resident community, or on the structure and function of the community." Other, more critical reviews of the same primary data dispute this conclusion, attributing it to a peculiar interpretation and a restricted reading of the data (16,23). Although debate continues, the ecological literature is replete with examples of communities perturbed by introductions, or of introductions invading and further disrupting environments previously disturbed. Most ecologists agree that successful introductions, while representing only a small portion of the total, usually have some effect on communities in the host environment.

Table 5-1.—Economic Significance of Insect Species Introduced to the United States, 1640-1977

Estimated total native insect fauna	104,000	species
Total insect introductions (1 percent of native fauna)	1,379	
Beneficial introductions:		
Deliberate	153	
Accidental	134	
Total	287	(20.8%)
Economically unimportant introductions	290	(21.03%)
Minor pests introduced	566	(41.04%)
Important pests introduced:		
Expected to be pests	80	(5.8%)
Not expected to be pests	156	(11.31%)
Total	236	(17.11%)

SOURCE: R.I. Sailer, "Our Immigrant Insect Fauna," *ESA Bulletin* 24:3-11, 1978.

Introductions into natural systems are most often successful where the host system has been previously disturbed (i.e., by human action or natural catastrophe such as volcanic eruption, severe storms, or floods) or is a "simple" or "disharmonic" system such as those found on oceanic islands. Introductions with the most serious consequences usually involve host systems or organisms that meet one or more of the following criteria (45):

- **Environments lacking potential predators**, natural competitors, or similar checks are vulnerable to disruption. Known examples of such disruption include herbivores (e.g., goats) introduced to islands, or generalist organisms that feed or prey from high positions in a food chain, and are thus unlikely to be checked by predation themselves (e.g., mongooses, rats, or cats).
- **Ecological generalists**, or species that can exploit a variety of resources for differing requirements during their life cycles, are particularly capable of invading and colonizing. Examples include organisms that can produce offspring by laying eggs in many different substances, and predators or parasites that can prey upon or parasitize a variety of prey or hosts.
- **Disturbed communities**, or relatively "simple" or genetically homogeneous communities, are especially vulnerable to any invading species that might exploit available resources more effectively.

These principles were abstracted from studies of natural systems. Although they illustrate situations that are most likely to result in problems, and therefore to be avoided where possible, their applicability to the majority of planned introductions is quite limited.

Introductions of Agricultural Varieties

Most of the introductions of genetically engineered organisms planned for the foreseeable future are intended for agricultural settings. In such systems—with a long history of introductions of new crop cultivars, animal varieties in husbandry, and microbes either for biocontrol of plant pathogens or inocculants for nitrogen fixation—a significant body of experience exists that is more directly relevant, with better developed control measures available, than is the case for introductions of exotic species.

The Agricultural Research Service of the U.S. Department of Agriculture (USDA) maintains a Plant Introduction Office that compiles data on all plant material brought into the United States. Since its inception in 1898, the office has recorded over 500,000 different plant samples (species and varieties). From 1982 to 1986, an average of 8,270 new accessions per year were added to USDA records (65). Over 90 percent of these were of foreign origin, and the majority (over 80 percent) had no measurable environmental or economic impact. About 10 percent of the introductions have proven to be valuable crops. A small number have become serious pests, such as Johnson grass, water hyacinth, and veronica.

Agricultural introductions of plants are susceptible to a variety of control measures, such as herbicides, tilling, or crop rotation, in cases where field performance is not acceptable. A plant is not likely to escape, and the propensities for hybridization with adjacent weedy species are well known (10), easily reviewed, and often subject to existing methods of mitigation and control (7,8).

Genetically engineered agricultural organisms are more likely to differ from parental strains in only one or a few genes, and to be introduced into a familiar environment, than they are to resemble introduced exotics, which usually consist of a completely new genome in a novel environment (see table 5-2). The small number of genes changed in most planned introductions should not be overinterpreted as grounds for reassurance, however, because in some cases single gene changes are known to have affected the virulence or host range of a parasite or pathogen (22,28, 32,56). Although this is of concern, such changes are not common, and engineered organisms are no more likely than nonengineered organisms to be susceptible to them. Indeed, recombinant DNA techniques enable engineered organisms to be changed from parental strains with respect to the genes controlling only one trait at a time. They are therefore likely to differ from parental strains less, and more precisely, than new varieties or cultivars produced by historical methods of hybridization and crossbreeding. This should permit a quicker understanding of any such shifts in host range or virulence affecting engineered organisms than was possible before, permitting in turn the earlier application of mitigation or control measures.

The introduction of selected microbes for agricultural purposes has been carried out for nearly a century (18,31). Substantial literature exists on the ecology of microbial plant pathogens and their introduction for biological control purposes (5,24, 48,62,64,67). Although uncertainties remain, this experience gives good reason to expect that planned introductions of genetically engineered microbes can be carried out safely.

All this does not mean, however, that there will be no problems with planned introductions of genetically engineered organisms. Indeed, in the long term (10 to 50 years), unforeseen ecological con-

Table 5-2.—A Comparison of Genetically Engineered Organisms and Exotic Species

Characteristics	Exotic organism[a]	Engineered organism[b]
No. of genes introduced	4,000 to >20,000	1 to 10
Evolutionary tuning	All genes have evolved to work together in a single package	Organism has several genes it may never have had before. Most likely to impose a cost or burden.
Relationship of organism to receiving environment	Foreign	Familiar, with possible exception of new genes

[a] "Exotic organism" is used here to mean one new to the habitat.

[b] "Engineered organism" is used here to mean a slightly modified (usually, but not always via recombinant DNA techniques) form of an organism already present in the habitat.

SOURCE: Office of Technology Assessment, 1988.

sequences of using recombinant organisms in agriculture are not only likely, they are probably inevitable. But it is crucial to put this into perspective: **It is difficult to describe a credible scenario that will lead to a problem that is different in kind from the problems created by, and grappled with, in past agricultural practices.** And while the adequacy of current regulatory policies in dealing with existing agricultural practice may deserve examination, **planned introductions of genetically engineered organisms do not appear to bring with them such potentially new problems that they require entirely new regulatory approaches or more stringent review.**

In summary, for the majority of planned introductions of genetically engineered organisms presently being contemplated, the **experience with new agricultural varieties is a better model of what can be expected than is experience from introduced exotic species. While there are grounds for caution, at least in the near term this experience offers more reason to be reassured than alarmed.**

POTENTIAL IMPACTS ON POPULATION OR COMMUNITY STRUCTURE OR INTERACTIONS

Much of the concern over planned introductions of genetically engineered organisms stems from the difficulty of predicting reliably the consequences of any particular ecological perturbation. In all cases, the ability to predict depends on the degree to which the organisms and the initial conditions involved are understood, and in some cases this is considerable. But the complexity of ecosystem processes and the numbers of different species even in simple systems means that there will always be uncertainties. Nevertheless, laboratory studies, greenhouse and small-scale field tests, and historical experience all suggest it is possible to anticipate the likely consequences of introductions planned for the near future (over the next 5 years).

The potential ecological impacts most likely to be noticed early involve effects on indigenous organisms. Such effects are more likely to be seen at the population or community level than at the level of ecosystem process. Engineered organisms that might cause such effects may be grouped into four categories (29):

1. slightly modified forms of native organisms;
2. organisms existing naturally in the target environment but requiring continual supplemental support;
3. organisms that exist naturally elsewhere in the environment, but that previously have not reached the target environment (or have reached it only at low levels); and
4. genuine novelties.

Slightly modified forms of indigenous organisms have a relatively high (but still low) probability of competing effectively with natives and thus of perturbing populations of closely related or conspecific individuals. Most deliberate releases for the foreseeable future are likely to be of this sort. Despite some exceptions, it remains true that **the majority of mutations or genetic changes are more likely to have negative than positive consequences for the competitive abilities of engineered organisms.** Thus researchers working to produce organisms for planned introductions are generally more concerned about the problems of enhancing the competitive abilities of engineered organisms so that they will survive to perform their intended functions. When genetic changes are directly aimed at enhancing competitive or survival abilities the review process should explicitly recognize this, analyze the selective forces involved, and review the potential implications.

Organisms existing naturally in the target environment, if they require continual supplemental support, are even less likely to cause negative effects. The need for continual supplementation provides an effective means of control: stop the supplementation, and growth or function of the introduced organism ceases. It would be logical for planned introductions of this sort to be most common in agricultural settings, but few are anticipated. Researchers and farmers are trying to minimize the need for continual replenishment (e.g., fertilizers for crop plants), not increase it. This desire is one of the factors that has driven agriculture to exploit biotechnology, in the hope that products can be engineered to be easily managed and self-sustaining once introduced. The need for continual supplementation might be exploited, however, to help control certain applications. But it must be realized that natural selection would be continually operating in ways that would decrease the effectiveness of this type of control, favoring genetic variants free of the imposed limitation.

The introduction of organisms that exist naturally elsewhere, but that have not previously reached the target environment, presents the highest probability of disrupting a community or constellation of species in a given area. This category bears the most similarity to the introduction of exotics, and is most likely to result in what have been called "cascade effects" (discussed in the section on insect communities). And although most introductions do not result in such severe effects, dramatic and far-reaching disruptions of community structure can take place. Especially here, though, perspective is important. Although some planned introductions are indeed intended to allow growth in environments new to the organism, appropriate regulatory oversight should minimize risks. One example of a beneficial introduction of this kind would be specific insect predators as biocontrol measures aimed at severe pests. In the past, biocontrol introductions that have been preceded by critical review have often been remarkably successful, with negative consequences rare, but sometimes substantial (25,44).

The effects of genuine novelties are the most difficult to predict because directly relevant experience is lacking. One of the chief problems here is the lack of agreement on what would constitute a genuine novelty. Some contend that inserting a gene into an organism that would thus obtain an entirely new function or property, as for example, Monsanto's *Bacillus thuringiensis* (BT) toxin containing pseudomonad, would constitute a novelty. There is no general agreement on this, however, worthy arguments being raised by both sides. Fortunately, few planned introductions envisioned in the near future fall into such disputed categories and it is broadly agreed that, for the foreseeable future, most engineered organisms will not be truly novel.

A clearer picture of the potentially negative consequences most likely from planned introductions can be gained from a review of the types of introductions anticipated in different categories of communities.

Plant Communities

The largest number of modifications to plants planned for introduction in the foreseeable future involves the insertion of genes to provide herbicide resistance. Most of the major companies working in agricultural biotechnology are mounting efforts in this direction, studying crops for which herbicides are useful in restricting competition from weeds. Such crops include tobacco (more valuable, however, as a well understood and malleable experimental system than as an end product for agricultural use in and of itself), tomatoes, corn, rape, soybeans, and cotton.

Introducing herbicide resistance genes into plants may bring ecological as well as economic benefits by increasing the use of safer herbicides and allowing their more precise administration. Success could lead to significant increases in market share for particular herbicides, some of which, however, are associated with significant environmental disadvantages.

Most of the herbicides to which resistance is being engineered (e.g., glyphosate or sulfonylurea) are environmentally "soft"—in other words, they are not highly toxic to humans or vertebrates. In many cases they affect only plants, since their target sites are metabolic pathways unique to plants. Some of these herbicides degrade within days or weeks to benign end products such as carbon dioxide and water. Work is also being done on inducing resistance to longer lasting, or more toxic, herbicides, such as atrazine. Successful research in such programs might well lead to increased use of some herbicides with less desirable characteristics. The particular cost/benefit estimates will vary with each herbicide and its qualities. Consideration of such factors might be more appropriate in reviews for product licensing than in the regulation of field tests, but could be included at any stage.

The major concern with herbicide resistance genes is that they might spread into related, weedy species, most likely through sexual reproduction (10). The potential drawbacks are obvious: Worldwide economic losses to weeds are still measured in billions of dollars per year. While this is a greater problem outside the United States, it is neither a new problem nor one unique to genetically engineered plants. In many cases such existing problems are managed by rotating either crops or herbicides.

Researchers are also trying to insert pest resistance genes directly into plants, which could reduce the need for pesticides. Several companies are pursuing such applications, but the principles are exemplified by the efforts of Rohm & Haas. Successful field tests have already taken place (see ch. 3 and app. A) with tobacco plants engineered to carry the delta-endotoxin gene from BT.

Different varieties of the toxin (derived from different strains of *B. thuringiensis*) are poisonous to the larvae of some herbivorous insects, primarily certain Lepidoptera (moths and butterflies) and Coleoptera (beetles). The toxin's action depends on fundamental biochemical characteristics (pH, membrane permeability, etc.) of the larval digestive tract in these insects. As the larvae mature, and their digestive tracts develop, they become less sensitive to the toxin. Young larvae are most sensitive.

The field test showed that the delta-endotoxin gene is expressed in engineered tobacco plants. The toxin produced in plant tissues conferred essentially complete protection against predation by the tobacco hornworm *Manduca sexta*. This caterpillar is a common agricultural pest on a variety of crops, and several companies are working on protecting different crop species by this or a similar technique.

There appears to be little, if any, cause to be alarmed at the direct consequences of this compound being present in large quantities in the environment. The BT toxin has been used in various formulations since the first product was licensed in 1962 (66). As indicated in chapter 4, it is presently available in 13 different formulations (e.g., powder or soluble concentrate) in over 410 different registered products. Total quantities that have been administered are in the hundreds of thousands of tons, with ill effects on humans, vertebrates, or nontarget organisms virtually unknown. (For one of the rare citations of a human problem associated with BT use, see (47).)

Yet there is still some reason for concern. Large-scale applications of toxin-containing plants or novel microbial delivery vehicles might promote the evolution of insect resistance to this currently safe and effective agent by increasing the distribution and persistence of the toxin in the environment. Delta-endotoxin is extremely effective against its target organisms. Evolutionary biologists would say it exerts a strong selection pressure. This means that given persistent exposure to the toxin, particularly in sublethal concentrations, target organisms could be expected eventually to evolve tolerance or resistance to delta-endotoxin through natural selection.

Because the toxin is so unstable, and sporadically used, it generally does not persist long enough for resistance to evolve. The BT toxin is a biodegradable protein, sensitive to temperature, moisture, and especially to ultraviolet (UV) light, which causes its rapid decomposition. Biotechnology could change this by packaging the toxin in new vehicles (e.g., the Mycogen product; see app. A) placing it in new locations (e.g., inside host plant tissues, as in the Rohm & Haas product) or both (e.g., the Monsanto product) where it would be protected from degradation. The toxin could persist in the environment long enough to allow natural selection to produce resistant insects. This scenario is supported by at least one report of evolved resistance to BT toxin (34), in which seeds stored in silos are dusted with BT. In this setting, where the toxin is protected from UV degradation, resistance in the insect pest has been observed to evolve rapidly, and in more than one instance.

This problem is less one of product safety, however, than of useful product life. The declining agricultural market for BT in recent years, as it has been replaced by promising new compounds like the synthetic pyrethroids, makes it unlikely that loss of BT as an agricultural pesticide would lead to an increased reliance on older, more dangerous chemical pesticides. But a consideration of possible responses to a potential problem such as this is illustrative.

Because the evolution of resistance is a product of selection pressure created by the chronic presence of the toxin, either when or where it is not needed (e.g., in roots or stems, in addition to the leaves that are eaten), the problem might be solved by limiting toxin distribution to the times and places it is really needed. One way to do this would be to limit the expression of the BT gene to only the tissues subject to damage by herbivorous insects. The system could be refined further by making gene expression inducible, so that toxin production in the plant tissues is triggered by damage caused by herbivorous insects (27). This strategy would require the insertion of precisely controlled regulatory sequences along with the BT toxin gene, an approach that is beyond the reach of current techniques. But with increased understanding of the mechanisms of gene regulation, it may be possible in the near future (21).

A second approach, while not so obvious, employs tactics that can be used effectively and immediately, though the logistics of this approach may complicate existing methods of planting and harvesting somewhat. It draws upon studies in game theory of evolutionary stable strategies (12,13,14,58). Some biologists who have studied the relationships between pathogens or pests and their hosts feel that pathogens or pests may adapt more quickly to the defenses of agricultural crops than to those of hosts in the natural environment (1,32). The key seems to lie in the differences in genetic variation in the two populations. Variation is higher in natural than in agricultural populations, because the latter often involve huge areas of genetically homogeneous host plants. The selection pressures in agricultural systems are more often even and continual, in marked contrast to the spatial, temporal, and other variables that complicate selection pressures in the natural environment. These even, continual selection pressures are much easier to adapt to. Thus, anything that could be done to increase the genetic variation (at least in the genes controlling pest defenses) of the host population would likely lead to a slower adaptive response in the pest.

Increasing genetic variation in agricultural populations could be accomplished by mixing genetically pest-resistant strains of crop plants with strains not so protected. Modeling studies show that as simple a change as planting a mixture of 50 percent resistant and 50 percent unprotected seed in place of 100 percent resistant seed would substantially extend the time it takes for the pest to mount an adaptive response (32). The same principle also applies to other host/pathogen relationships, such as wheat/rust or corn/blight, or integrated pest management in general. Indeed, the relationship could be extended to planting crops that vary with respect to their tolerance to environmental factors (drought, cold) in areas where wide annual fluctuations in these parameters occur. The net effect could well be an increase in crop yields or productivity, although most often there is a trade-off between yield and the degree of resistance or tolerance. Just as complex communities seem best to resist the perturbing effects of introduced species (17), so might genetically complex agricultural systems better resist many types of potential environmental challenge.

Other types of genetic modifications to plants, such as altering photosynthetic pathways to increase efficiency and production rates, or modifying seed components to resist insect predation and loss during storage, may well have environmental effects of an unforeseen nature. But much larger changes in photosynthetic rates or biomass productivity can be achieved more easily by planting different crops, something that takes place constantly without review for potential environmental impacts. It hardly seems logical, therefore, to review engineered plants for such an effect when traditional agricultural crops are not subjected to such review.

Insect Communities

A great deal of research in genetically altered plants and microbes focuses on ways to combat insect pests—a major destroyer of both field and stored crops. Relatively little work is presently being done with insects per se. Some plant modifications for controlling insects were discussed in the preceding section. This section describes two representative modifications to insect-targeted microbes: those intended to reduce crop losses to the black cutworm, and modifications to viruses that parasitize certain insect species.

Significant corn crop losses each year are attributable to one pest species—the black cutworm. This lepidopteran larva feeds on the roots of corn plants. To combat this pest, researchers are exploiting both its sensitivity to the delta-endotoxin from *B. thuringiensis* and its narrow host range of corn roots. Their work involves engineered versions of the common bacterium *Pseudomonas fluorescens*.

Some strains of *P. fluorescens* live in the rhizosphere formed by the interaction between corn roots and associated filamentous fungi. Scientists at Monsanto have used a specific transposable element, Tn5, to insert the delta-endotoxin gene into the chromosome of this bacterium. This transposable element is not common in nature (6,41). Monsanto scientists have disarmed it in two independent ways to make further movement after insertion unlikely (41). In addition, frameshift mutations were inserted to further decrease the possibility of horizontal gene transfer due to reversion or complementation.

Greenhouse tests suggest that if corn seeds are coated with the altered *Pseudomonas*, the bacterium will colonize the emerging roots upon germination. Preliminary results also indicate that the engineered bacteria do not disperse significantly beyond the rhizosphere habitat in which they are naturally found, nor do they seem to persist beyond the end of the corn growing season. All these factors, coupled with the known, safe record of the BT toxin, suggest that this environmental application is safe enough to be field tested on a small scale. Such a test would measure survival, dispersal, and efficacy under realistic field conditions, and could be expected to provide useful lessons for other microbial applications.

Another early microbial application aimed at insect pests uses the host specificity of a class of viruses known as baculoviruses. These have relatively narrow host ranges, usually one or a few closely related insect species. Different viruses are specific for different pests, including the cabbage looper (a moth larva) and the pine sawfly, both of which threaten agriculture and forestry in the United Kingdom.

During 1986, researchers at the Institute of Virology in Oxford concluded a preliminary field test of a cabbage-looper virus. To track the released virus, the researchers inserted specific, marker DNA sequences into the engineered organism. Similar tests are planned for a virus specific for the pine sawfly.

Scientists hope that learning more about the genetics of host specificity in such viruses will enable them to target viruses precisely to specific pests. But the difficulties of tracking a virus in the environment, the possibilities for dispersal, the ability of viruses to remain infective for long periods, and the possibility of mutations disrupting the host range of released forms must be considered before wholesale applications are undertaken. When asked about the degree of risk attending such work, one researcher responded,

> My guess is no problem, but there are a variety of constructs possible, and some could have broader effects than desired . . . One can consider many scenarios. In the worst case (also the most improbable), the situation could not be corrected (36).

One of the least predictable effects of environmental alterations has been termed the "cascade effect." Such effects are not likely to be common consequences of planned introductions, and quite unlikely to be associated with small-scale field tests. But they are known to have been associated with some large-scale environmental activities in the past, so a brief description is in order. The term "cascade effect" describes a community disruption in which a perturbation in numbers (or even loss) of one species triggers reverberations throughout the community. These effects could alter predator/prey relationships or significantly change community structure even if ecosystem processes themselves are not altered.

Cascade effects can be both surprising and counterintuitive. One example can be found in the case of a World Health Organization program to control insect pests. The program used large-scale applications of DDT in Borneo to kill house flies. The local lizard population, however, could not distinguish between flies that died of natural causes, and were safe to eat, and those that succumbed to the spraying program, and therefore were not. Large numbers of geckos ate the flies and died, as did cats that ate the poisoned geckos. The reduction in the cat population allowed rat numbers to rise. Stored food supplies suffered, and the increased numbers of rats brought an increase in the numbers of rat pest species. The result: an outbreak of bubonic plague, a public health problem that would not normally be expected to result from a program of insect eradication (30).

Although such chains of events are almost wholly irrelevant to considerations of field-test safety, they should be kept in mind when the large-scale applications that will follow are contemplated. It should also be noted that the negative consequences of the house-fly eradication program resulted from an accumulation in the food chain of an enduring, toxic compound. Widespread applications of genetically engineered organisms are generally expected to reduce reliance on compounds of this sort. Also, most other examples of cascade effects involve perturbations of natural, not agricultural systems. Great care must be taken in extrapolating from nature to the artificial environments found in agriculture. Nevertheless, large-scale applications of insecticides have sometimes increased the numbers of insect pests when beneficial insects are eliminated along with the pests (35).

Examples of cascade effects resulting from use of a chemical pesticide bear little relation to the problems most likely with planned introductions of genetically engineered organisms. They do, however, illustrate that complex relationships govern the interplay of organisms in ecosystems—relationships that might not be comprehended with cursory review, and that are not always immediately apparent.

Microbial Communities

Although some concern is raised over possible consequences of introducing engineered animals or plants, greater public apprehension is associated with possible uses of engineered microbes (ch. 3). Planned applications include microbes altered to prevent plant damage by herbivorous insects (as just discussed), protect crops from frost damage, increase nitrogen available to plants, and, eventually, to degrade toxic wastes (covered later in this chapter). These introductions may take place either in limited agricultural settings or in broadcast environmental applications.

The greater concern over planned microbial introductions has less to do with a higher probability of risk than with greater uncertainty about some factors in microbial ecology and the requirements of monitoring or tracking that are peculiar to microbes. Extensive experience with past microbial introductions does not suggest that potential problems are worse than those associated with plants or animals. But few of the microbes living in soils can be cultured in the lab, and little is known about them or their relationships beyond the broad outlines of morphology and apparent function. Microbial ecologists do know, however, that there is a high degree of functional redundancy among members of microbial communities, a redundancy that should act to mitigate any general consequences of perturbing a particular microbial population, although some limited communities (e.g., degraders of lignin) may be less protected by such redundancy. Furthermore, a substantial literature suggests that microbial systems are resilient in the face of perturbations, and that it is often difficult to produce a measurable impact, even by design, on such populations.

Developing environmental applications with microbes affords certain advantages over working with plants or animals. Enormous populations (numbering in the billions) can be studied over hundreds of generations on a rapid time scale. On the other hand, microbes present some unique problems. They are microscopic, and the techniques for tracking their movements, distribution, and numbers are often more involved, or more tedious and expensive, than for plants or animals.

Microbes, particularly those in soil, can be difficult to detect. The most sensitive available techniques for sampling populations, based on detecting microbial DNA, are difficult or impossible to apply if the DNA to be tested is taken from fewer than a thousand cells. Smaller numbers—as low as 100 cells per cubic centimeter—may be assayed if bacterial colonies are cultured from naturally occurring individuals and screened with techniques that rely upon resistance to specific antibiotics, or other biochemical markers. This assumes, however, that the species can be grown in the laboratory.

Bacteria can persist in a microbial community at levels undetectable by any existing sampling technique. These levels can rise rapidly under favorable conditions, so the inability to detect a microbe does not mean that it is absent from an environment. Microbial species are enormously abundant—upwards of a thousand different species may be found per square yard of land surface (9). A single species may encompass from 5,000 to 20,000 genetically different strains or varieties, varying in their adaptive qualities and ecological requirements.

Microbes are also ubiquitous, found in every terrestrial environment and habitat in which they have been sought, including such hostile and unlikely sites as hot springs and subterranean aquifers (11,20). Perhaps 90 percent of these species cannot presently be cultured in the laboratory, making them difficult to study. Despite such obstacles, the ecological questions raised by the environmental release of genetically engineered microbes are not intractable, and a great deal of relevant historical experience can be studied.

Genetic alterations to microbes for specific environmental purposes are many and various. This section outlines two not covered elsewhere in this report. The first—the application of "ice-minus" bacteria to crop plants to protect against frost damage—involves what was one of the more contentious of the early applications for permission to field test, though for reasons other than the scientific questions involved (see ch. 3). The second, involving the inoculation of crops with enhanced nitrogen-fixing bacteria, has been less controversial.

The technical details of the ice-minus application are simple. Some bacteria contain, as a component of the cellular membrane, a protein encoded by a single gene in the bacterial chromosome. This protein can act as an efficient nucleus for the formation of ice crystals on the surfaces of plant leaves or blossoms where the bacteria live. Without such nuclei, water does not generally freeze at 32 °F. Rather, it supercools to between 5 and 10° below the "normal" freezing point before the formation of ice crystals begins to take place.

Photo credit: Peter Forde, Advanced Genetic Sciences

Advanced Genetic Sciences Researcher Julianne Lindemann spraying "ice-minus" bacteria on strawberry plants in test plot on April 24, 1987. Protective clothing was required by the California Department of Health Services. Note reporters and onlookers in immediate background where coffee and donuts were consumed without hesitation.

Agricultural losses to frost damage each year are variable but significant. Some estimates place the average annual loss at $1.6 billion in the United States, and $14 billion worldwide (15). Most of this loss results from frosts that take place near harvest time, or near flower-budding time in spring. Part of it might be avoidable if some protection could be provided against snap frosts, as opposed to hard freezes, because hard freezes are uncommon during the growing seasons of most crops.

Photo credit: Peter Forde, Advanced Genetic Sciences

Advanced Genetic Sciences Researcher Trevor Suslow spraying "ice-minus" bacteria on strawberry plants in test plot in December 1987. Note minimal protective gear.

Protection could be afforded by deleting the "ice-nucleating" gene (or an essential part of it) from the major bacterial strains living on the surfaces of particular plants (potatoes and strawberries, in the first field tests). These altered bacteria would then be inoculated on the plant surfaces to be protected, in the hope that they would colonize them and replace the naturally occurring bacteria (designated ice plus, or INA+, for ice nucleation active).

Naturally occurring populations of ice-nucleating bacteria contain low numbers of individuals that are ice-minus. The phenotype can be produced by any mutation that inactivates the production of the critical protein, and thousands of such mutations are possible. Many could produce ice-minus bacteria in natural populations. The primary genetic difference between these naturally occurring ice-minus bacteria and those produced with recombinant DNA techniques is that the latter are produced by a specific technique that yields a consistent, precisely characterized, identifiable genetic deletion. Such consistency and identity is not a characteristic of the naturally occurring strains. These and other factors led both the National Institutes of Health and the Environmental Protection Agency to approve applications for preliminary, small-scale field tests of the bacteria, which were successfully completed in 1987.

Some scientists suggested a worst-case scenario that sounds like a cascade effect (42). According to that scenario (which presupposed large-scale applications, not small-scale field tests), large-scale agricultural applications of ice-minus bacteria might reduce the atmospheric reservoir of ice nuclei. These atmospheric nuclei are critical for precipitation, providing particles around which ice crystals can form so that droplets can grow large enough to fall as snow or rain. Dirt and dust can act as sources of ice nuclei, but vegetable or plant material is a better source (53,54). Another major source for ice nuclei is marine phytoplankton, living in the top layers of oceanic waters (50,51). The sources of ice nuclei important to local precipitation are generally local (52,55), though long-distance dispersion of bacteria is known to be possible, if not common (19), and less likely for good nucleators than nonnucleators.

Some scientists asked if reducing atmospheric concentrations of ice nuclei could affect local rainfall, and perhaps even global weather, reducing or redistributing patterns of precipitation. They pointed to data suggesting that overgrazing in the Sahel may have exacerbated drought conditions in Sahelian Africa (43,49). Overgrazing reduces plant biomass in an ecosystem, thus reducing the suitable habitat for ice-nucleating bacteria, and reducing the numbers of bacteria. Such a cascade effect may have contributed to decreases in rainfall downwind of deforested areas.

Seeking to assess the likelihood of this scenario, OTA commissioned two analytical studies by groups taking slightly different approaches to the problem (4,60). Both groups made assumptions to produce a worst case scenario. Both concluded it is unrealistic to expect any significant negative impact on global climatological patterns from large-scale agricultural applications of ice-minus bacteria. The likelihood of local changes in precipitation patterns or densities is perhaps slightly higher, they concluded, but still extremely low. Indeed, to put the question in the appropriate context, the potential for negative environmental consequences from large-scale applications of ice minus bacteria should be compared with the potential for such consequences from other strategies for reducing or eliminating INA + bacteria in agriculture, such as the use of chemical bactericides. Several significant uncertainties in these analyses could be resolved, however, by further research and experimental studies.

In another planned introduction of engineered microbes, rhizobial bacteria are being studied in an effort to increase nitrogen fixation in soil, and thus to decrease reliance on costly fertilizers. Scientists at BioTechnica International (Cambridge, MA) have engineered a strain of *Rhizobium meliloti*, symbiotic on alfalfa roots, to increase its production of nitrogen. This has been accomplished by increasing the number of promoter sequences that regulate the activity of genes involved in nitrogen fixation metabolism. Greenhouse tests suggest possible increases of available nitrogen as high as 17 percent, and though field tests were originally planned for 1987, they were later postponed until 1988. A long history of agricultural inoculations with different varieties of nitrogen-fixing bacteria suggests that far-reaching or negative environmental consequences are quite unlikely, though this question is explored in more detail later in this chapter.

Some individuals believe other approaches to nitrogen fixation may lead even more quickly to practical improvements. This feeling stems partly from the fact that nitrogen fixation is an energy-intensive process. Sufficient energy may not be available under most conditions to sustain appreciable increases in nitrogen fixation by microorganisms residing on or around root surfaces. It is also true that bacteria that are good nodulators or colonizers of roots also tend not to be the most efficient fixers of nitrogen, though there is tremendous variation among naturally existing strains.

Improving the energy efficiency of these bacteria is, of course, one object of research. But re-

Photo credit: John Albers

BioTechnica International test plot for nitrogen-fixing alfalfa/rhizobial bacteria system in Pepin County, Wisconsin.

search efforts might profitably be invested in improving the ability of nitrogen-fixing microbes to withstand the stresses encountered in their rhizobial environments and in the processes of storage and agricultural inoculation. Most bacteria now marketed as inoculants fare poorly under these conditions, especially in the tropics, with the result that most attempted inoculations deliver only a small fraction of the intended microbes to the application site. Research in these areas might dramatically improve the success of inoculation techniques while effectively exploiting the existing reservoir of variation among naturally occurring rhizobial bacteria in different environments (2,3).

Aquatic Communities

Work on altering aquatic community species is being pursued in a number of areas. Potentially fruitful lines of investigation involve fisheries biology and aquaculture. At least two different research groups are exploring methods to enhance the production of "trophy" salmon in the Great Lakes region and the Pacific Northwest (see app. A). Under a flexible definition of biotechnology, this research may qualify for consideration in this report, or it may not be considered to differ significantly from past fisheries practice.

The technique used to try to produce the trophy salmon is called "heat shock." During an early, sensitive stage of embryonic development, salmon eggs are briefly subjected to unusually high temperatures (38 degrees Celsius): temperatures high enough to disrupt some metabolic processes, but not so high as to kill the developing fish embryo. This environmental insult disrupts normal cell division, causing a spontaneous doubling of the chromosomal number in each cell of the developing embryo.

Chromosomal doubling triggers a series of physiological effects, starting with a disruption of the hormonal system. The fish fail to mature sexually and remain sterile throughout their lives. This frees them from the normal cycle of growth, maturation, sexual reproduction (spawning), and death that usually limits their lifespan. Because these fish fail to spawn and die, they are expected to continue to live and grow, eventually reaching record sizes.

Salmonid fish are among the top predators in whatever community they inhabit. So initially it may appear that the planned introduction of these fish would violate a cardinal principle mentioned earlier: Do not introduce polyphagous species, particularly if they occupy a high position in a food chain (45). But these fisheries are already managed by humans. Even under generous assumptions about the survival of released fingerlings, the resulting adults are unlikely to number more than several hundred among stocked populations that number in the millions (33). Furthermore, the same property that causes these fish to reach such large sizes prevents them from reproducing. It is impossible that any environment will ever contain these fish except through planned stocking. Careful monitoring of the fish's release and growth rates should help to avoid or correct serious unanticipated effects.

Other work with aquatic communities focuses on marine algae, to enhance the rates at which they produce substances useful in food preparation, such as carrageenan or agar (see app. A). Some aquatic algae have also been considered as potential agents to extract toxic compounds or heavy metals from aqueous solution (59). It might even be possible to exploit algae for mining or mineral recovery operations. But most of these applications involve aquatic plants that function in their environments as primary producers. Genetic alterations to improve or adjust their rates of activity could well affect other members of aquatic communities. Such possible effects are discussed in the following section.

POTENTIAL IMPACTS ON ECOSYSTEM PROCESSES

Ecosystems are enormously complex, and to reach a clear understanding of how they function is commensurately difficult. Their complexity stems from the many and diverse interactions within the numerous populations of any given species, and between the great numbers of different species that form any biological community. It is also due to the links between the physiological processes of organisms and the geological processes by which minerals are derived, distributed, and moved in the physical world. For example, the availability of minerals liberated from rocks is affected by living things (especially plants or microbes) as they influence rates of erosion.

As a way of reducing this daunting complexity to manageable proportions, some ecologists devote more study to the distribution and movement of a small number of vital elements and minerals than to the activities and attributes of living creatures. The most important of these vital elements are carbon, nitrogen, phosphorous, and sulfur. These elements are "rate-limiting." In other words, the amount of these elements in biologically accessible forms determines the total tissue of living organisms (biomass) that can be assembled in the ecosystem. (Phosphorous and sulfur are covered in detail in 9,22.) Carbon and nitrogen are discussed in this section.

Ecosystem processes are the vehicles by which these rate-limiting elements and their different molecular forms move, or cycle in ecosystems. The two major ecosystem processes are **nutrient cycles** and **energy flow**. These processes can be, and sometimes are, considered separately. This report will consider them together, and deal with them primarily in the language of mineral or nutrient cycles.

Every time an element is altered from one molecular form to another, or exchanged between different organisms, some chemical bonds are broken and others formed. Each of these actions either consumes or liberates energy. The integration of countless individual events of this sort is the immediate mechanism by which nutrients cycle and energy flows through an ecosystem.

The major factors driving ecosystem processes are the production of energy and the conversion of carbon into biological forms by photosynthesis, or carbon fixation. Where carbon is rate-limiting, it places an absolute upper limit on the biomass a system can support. Under such circumstances, competition for carbon is intense, as for example among soil microbes. Plants produce most of the biologically accessible forms of carbon.

Decomposers—organisms that degrade plant material—play the major role in moving carbon from one reservoir into another (e.g., from one plant, through decay, into another). Most decomposers are invertebrates (insects, nematodes, and so on) or microbes (bacteria and fungi), living primarily near the soil surface in what is termed the "litter layer" of detritus that accumulates from above, or just below, in the topsoil. Larger herbivores, or vertebrates, play a smaller role, usually assisted by an intestinal flora of symbiotic microbes.

Physical parameters, such as climate, soil quality, and available moisture, affect the distribution of both plants and decomposers. As a result, a range of ecosystems—different communities of plants and decomposers, associated with particular ecological qualities—has evolved. Table 5-3 illustrates several different terrestrial ecosystems and presents data indicating the patterns of carbon storage and movement in them.

Nitrogen is the nutrient most often cited as rate-limiting in terrestrial ecosystems. Although one form of nitrogen makes up nearly 80 percent of

Table 5-3.—Production and Decomposition in Six Ecosystem Types

	Mean NPP tons/ha/yr	Mean biomass tons/ha	Litterfall tons/ha/yr	Litter accumulation tons/ha	Decomposition k/yr
Tundra	1.5	10	1.5	44	0.03
Boreal forest	7.5	200	7.5	35	0.21
Deciduous forest	11.5	350	11.5	15	0.77
Grassland	7.5	18	7.5	5	1.50
Savannah	9.5	45	9.5	3	3.20
Tropical forest	30.0	500	30.0	5	6.00

Abbreviations: NPP = net primary productivity, the total amount of living material produced by the organisms in an ecosystem; ha = hectare; yr = year; k = fractional weight loss of litter material (a value of 1 means the rates of production and decomposition are equal).

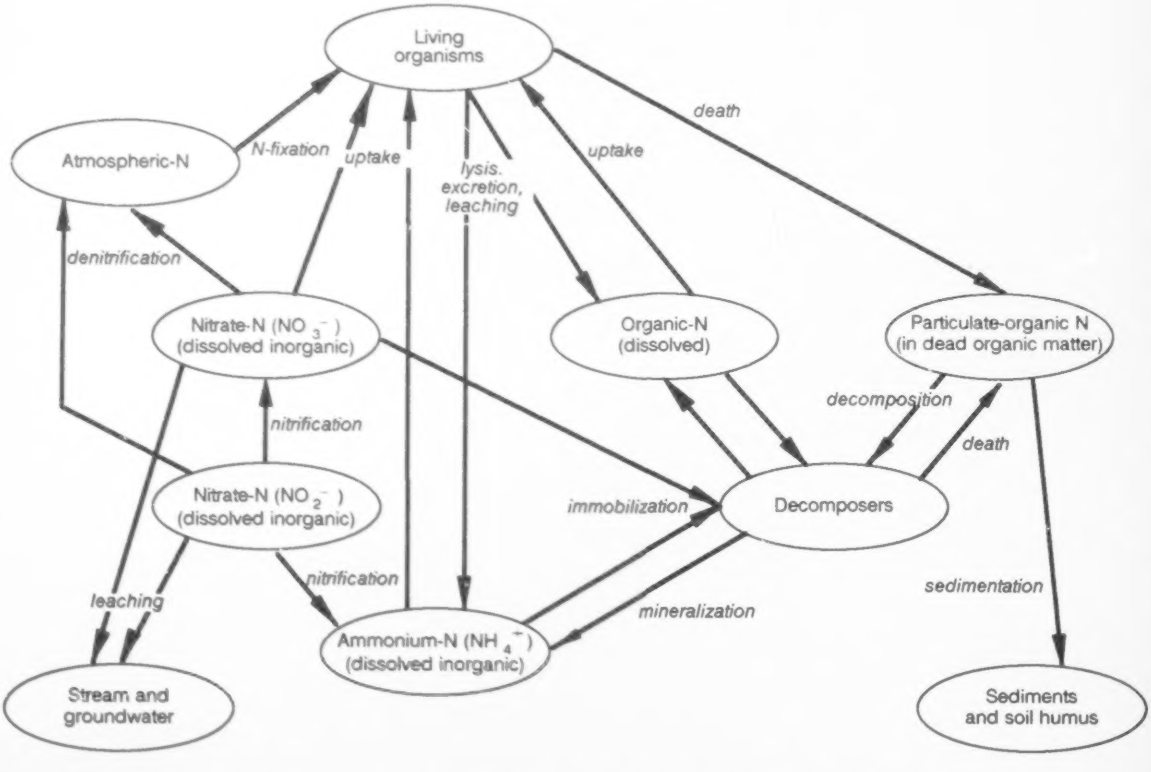
SOURCE: J.R. Gosz, C.N. Dahm, and P.W. Flanagan, "Ecological Impact of Genetically Engineered Organisms on Ecosystems," contract report prepared for the Office of Technology Assessment, U.S. Congress, 1987.

the atmosphere, biologically accessible forms are more limited.

The nitrogen cycle—illustrated in figure 5-1—is the most complex major nutrient cycle because the element can exist in so many qualitatively different molecular forms. Abiotic factors and the actions of living things influence the production and persistence of these forms.

While plants can absorb gaseous nitrogen or nitrogen dissolved in water through their roots, microbes provide the major pathway for nitrogen incorporation into living material. Specifically, rhizobial bacteria are responsible for nitrogen fixation in agriculture. These microbes live in very close association with the roots of certain plants (legumes), often in small granules or nodules attached to the roots. In essence, they deliver fixed nitrogen directly to the host plant. The biochemical pathway resulting in nitrogen fixation is large, complex, and energetically expensive to operate, involving at least 17 different structural genes, with associated regulatory sequences. This genetic package exists in similar form in all rhizobial bacteria, an evolutionary conservation suggesting that the pathway is quite important to the bacteria. Scientists trying to enhance nitrogen fixation face the formidable task of improving the performance of a genetic sequence that has been refined by natural selection for billions of years. Nevertheless, researchers are making the attempt, even trying to transfer the complex of nitrogen fixa-

Figure 5-1.—The Nitrogen Cycle


Living organisms
Atmospheric-N
N-fixation
uptake
lysis, excretion, leaching
uptake
death
denitrification
Nitrate-N (NO3−) (dissolved inorganic)
Organic-N (dissolved)
Particulate-organic N (in dead organic matter)
decomposition
nitrification
death
Nitrate-N (NO2−) (dissolved inorganic)
immobilization
Decomposers
nitrification
leaching
mineralization
sedimentation
Ammonium-N (NH4+) (dissolved inorganic)
Stream and groundwater
Sediments and soil humus

The nitrogen cycle: processes in terrestrial and aquatic systems. The microbially mediated processes include mineralization, nitrification, denitrification, immobilization, and N-fixation.

SOURCE: Office of Technology Assessment, 1988.

tion genes into plants to enable them to produce their own nitrogen without relying on associated bacteria.

If the nitrogen-fixing abilities of rhizobial bacteria were enhanced in such a way as to make the resultant increase in available nitrogen general throughout the host environment, rather than restricted to the host plant per se, a variety of environmental consequences might result. The potential consequences of such a successful application are listed here (22), in order of decreasing probability.

1. a) Increased leaf and shoot production in target plants
   b) Decreased root growth and biomass
   c) Increased decomposition rates in some systems
   d) Increased ammonification and nitrification in soil
   e) Increased competition on nonnitrogen-fixing plants and alteration of plant community structure, with reverberations throughout the associated ecological community
2. a) Decreased decomposition rates in some systems
   b) Increased potential for other element limitations, especially if 2a results
   c) Altered chemical exchange capacities and pH shifts
3. a) Decreased mycorrhizal associations (dependent on 2b)
   b) Shift from carbon-based plant defense to nitrogen-based defense (or from immobile to mobile defense compounds)
   c) Altered foliage tissue palatability and herbivory (dependent on 3b)
   d) Increased terrestrial denitrification rates and increased emissions of Nitrogen gases (dependent on 1d and 2c)
4. a) Increased leaching loss of nitrate
   b) Increased eutrophication of drainage water
   c) Increased algal production in receiving waters
   d) Increased carbon enrichment of aquatic sediments
   e) Increased anaerobic conditions in aquatic habitats
   f) Increased aquatic nitrification and denitrification rates and gaseous emissions to the atmosphere.

Some data suggest such consequences are not purely hypothetical, that under very special conditions they might actually be likely (63). Generally, however, such a concatenation of consequences rests on a series of assumptions. For most of them to occur, the introduced rhizobia would have to move beyond agricultural lands. They would then have to successfully nodulate, or colonize, wild plants, and provide substantially more nitrogen to those wild plants than is already provided by native rhizobia. This also requires that the host specificity of the introduced rhizobia would have to change genetically so they could infect the wild plants, and the introduced bacteria would have to be successful in nodulating the native plants in competition with native bacteria. As cited above, considerable literature and experience demonstrates the remote likelihood of each of these assumptions; to achieve all of them at once, the probability is exponentially less likely. Indeed, a review of these assumptions and potential events leads many experts on nitrogen fixation to believe it would be difficult to conceive of a planned introduction less likely to have negative consequences than introducing engineered varieties of nitrogen-fixing bacteria.

Ecosystem processes reflect the sum total of the actions of all living things in the system. Individuals concerned about planned introductions of genetically engineered organisms fear applications that might affect such fundamental processes as nitrogen or phosphorous cycles. And although basic changes to an ecosystem process would be difficult to accomplish, it might be easier to affect changes in rates of flow of some components through a portion of the process. By the time such effects were noticed, however, a community might already have been substantially altered. According to two researchers in this field,

> . . . the quality of an environment can change markedly with no significant change in gross measures of the rates of processes. Fish production in two lakes may be the same (as lbs./year) when one produces mostly rainbow trout and the other mostly carp, but no human would say that the two lakes are therefore indistinguishable with regard to fish (9).

Attempts to detect such changes in ecosystems have led to the study of so-called indicator species, species more sensitive than other organisms to environmental changes (40).

The use of indicator species requires the identification of species that are particularly sensitive to the environmental parameter of interest—for example, the presence of toxic compounds or the abundance of specific nutrients. Indicator species can give early warning of an impending change, allowing preemptive measures to be taken. Without such early warnings and preemptive action, the task of restoring a disrupted ecosystem is difficult, expensive, and slow, if even possible (38).

A better understanding of community relationships should make indicator species increasingly useful, especially in risk assessment and management. Nevertheless, any planned introduction that is likely to alter a fundamental ecosystem process should undergo careful scrutiny. None are planned in the foreseeable future.

Possibly of eventual concern, however, are applications intended to correct existing serious, generally recognized environmental problems, such as the presence of toxic chemicals. Naturally occurring microbes exist that can degrade many different, complex, toxic compounds—herbicides and pesticides, industrial solvents, wood preservatives, plasticizers, dyes, etc. (22,26). Biotechnology researchers are trying to enhance these natural degradative abilities in some microbes and introduce them into others. Success in such efforts could help significantly in decreasing the toxic waste problem that promises to be so difficult and expensive to resolve (61).

Cascade effects might be triggered by the structural similarities between naturally occurring plant materials and some of the complex organic compounds found among toxic wastes. If degraders were engineered and introduced without sufficient constraints upon their specificity, they could function as ecological generalists, breaking one of the foremost rules for introducing organisms into new environments. Communities of decomposers may also be more vulnerable to perturbations than most microbial communities, because the decomposition of lignin and cellulose (the structural components that make up from 50 to 90 percent of the biomass of higher plants) is carried out by a relatively narrow range of microorganisms. A disruption of the population dynamics of one species of decomposer, in a limited community of decomposers made up of only a small number of species, could conceivably have a significant impact on the community.

In contrast, many other microbially mediated processes are carried out by the members of much larger, more diverse, and therefore more buffered, microbial communities. Indeed, no existing evidence indicates that populations even of microbial degraders are subject to cascade effects following perturbation. Such scenarios are purely speculative. In fact, some researchers in this area feel the introduction of engineered microbes to help in the degradation of organic pollutants is sufficiently distant that it need play no role in current deliberations over the safety of planned introductions, although similar introductions of naturally occurring organisms are not at all uncommon.

Finally, it is important to keep the possible consequences of planned introductions of genetically engineered organisms in perspective. As one group of ecologists has concluded, "Our analyses and remarks to this point may give the impression that we are against the release of any genetically altered organisms into the environment. This is not our view. In fact, we are enthusiastic about the prospects offered by biotechnological solutions to environmental problems ranging from increased productivity of agricultural systems, to the control of pests and pathogens, and the removal of many chemical toxins from the earth's soils and waters. We further expect that many, if not most, environmental applications of biotechnology can be made into safe and productive ventures" (28). Risk assessment and management, discussed in chapter 6, are crucial to that undertaking.

SUMMARY AND CONCLUSIONS

The major concern of some scientists over the planned introduction of genetically engineered organisms stems from the potential for unexpected or unforeseen consequences; other scientists believe that the likelihood of such consequences is no different, or even lower, for engineered organisms than for varieties or cultivars produced by widely accepted methods. A variety of disruptions to local populations or to more general ecosystem processes could result, but do not seem likely to result from any of the planned introductions now contemplated. Experiences with past introductions of organisms into new environments provide some limited clues to the nature of those disruptions, but a much better analogy for planned introductions of engineered organisms likely in the near future is that with new crops or cultivars in agriculture.

Only a small fraction of introduced organisms have become pests or have significantly affected their new environments. These species—called colonizing species, or weeds—differ significantly in their adaptive capabilities from most genetically engineered organisms intended for planned introductions. For the near future, engineered organisms generally will differ from nonproblematic parental strains by only one or a few structural genes. There are, however, several examples of single gene changes affecting the virulence or host range of a parasite or pathogen. Although engineered organisms are no more likely than nonengineered ones to be susceptible to such changes, until sufficient experience is gathered caution is indicated.

Environmental disruptions stemming from the deliberate release of engineered organisms might take place at any level—from the local population to ecosystem processes of energy flow or nutrient cycles. Plant introductions seem least likely to result in problems because of the low mobility of plants and restricted horizontal gene flow between them. Animal communities, because of the greater mobility and higher propensity for gene transfer, seem slightly more vulnerable to disruption, though the probability still seems quite low. Furthermore, most anticipated animal applications involve livestock, suggesting that the potential for disruptions of natural communities is not great.

Most difficult to assess are applications involving microbes. Microbial mobility is low but dispersability is relatively high, as is the potential for gene transfer. Much remains to be learned about the composition and relationships among naturally occurring microbial communities (of which only a small portion of the members can be cultured in laboratories) before general methods of risk assessment will be available (see ch. 6). But a substantial body of experience suggests that microbial introductions are not likely to produce problems.

The least likely, but potentially most serious ecological impacts involve the disruption of ecosystem processes, such as energy flow and nutrient cycling. Such disruptions could be difficult to reverse and far-reaching in their effects. They could result as a consequence of engineering microbes to increase their capabilities as degraders, e.g., to enhance the decomposition of woody tissues containing lignin or cellulose, or to help in the cleanup of toxic organic wastes. In the latter case, however, effective control might well be exerted by managing the available supplies of carbon, which are likely to constrain degradation rates. The potential problems associated with such enhanced degraders must be weighed against the existing, serious problems associated with toxic wastes and the chemical technologies that are their source.

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Chapter 6

Risk Assessment

"The atomic bomb was the first breach in our innocent conviction of the beneficience of science. My concern is that genetic engineering not become the second."

Robert Sinsheimer
New Scientist, January 20, 1977

"There will be vast numbers of tests going on simultaneously at some point. We'll have to move past the stage when every one of these becomes a focal point."

Allen Dines
Philadelphia Enquirer, May 17, 1987

"I'm drawing up the Whole Risk Catalog. Under D, I have dogs, doctors, dioxin. Where do I put DNA? Very low."

James D. Watson
The DNA Story: A Documentary History of Gene Cloning
W.H. Freeman & Co., 1981

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Chapter 6

Risk Assessment

During the 1970s, public concern about the effects of technology on the environment and human health heightened. As mounting scientific evidence confirmed that many chemical substances produced adverse effects (both acute and chronic) on humans and the environment, the government established programs to control potential hazards. Protocols and procedures—methods for the assessment of risks—were developed to enable regulators to evaluate potential hazardous substances. (See ch. 3 for discussion of the regulatory regime and public perceptions of these issues.)

Risk assessment of recombinant DNA technology is not new, nor is risk assessment of biological products. Nevertheless, questions concerning risk assessment of planned introductions of genetically engineered organisms into the environment recently have become the subject of debate. Some scientists perceive some risks associated with intentionally introducing these organisms as unique. Others believe introducing recombinant organisms is no different (and probably safer, because scientists constructed them with precision) than introducing nonrecombinant organisms, which has occurred for millennia.

Are there unique risks in environmental applications of genetically engineered products? Is additional information needed to assess the risks accurately? Can existing assessment methods (e.g., for other biological risks, or for chemical risks) be applied to the planned introductions of genetically engineered organisms? Are there areas where available baseline data are sufficient?

RISK ASSESSMENT V. RISK MANAGEMENT

Risk assessment is the use of scientific data to estimate the effects of exposure to hazardous materials or conditions. Derived from a factual base, risk assessment identifies and characterizes the magnitude of potential adverse affects —either their quality or quantity. It is a separate and distinct process from risk management.

Risk management is the process of weighing alternatives to select the most appropriate regulatory strategy or action. It integrates the results of risk assessment with technical, social, economic, and political concerns. Carried out by regulatory agencies under legislative mandates, risk management is a decisionmaking process requiring value judgments that compare potential risks and benefits, and determine the reasonableness of control costs. **Benefits are part of the calculus of risk management**. For example, risk management of biotechnological products involves comparing their benefits and problems against those associated with products they are designed to replace.

Risk management must also consider the economic and social costs of regulation. These include the burden of paperwork associated with regulation, and the costs of mitigation and control technologies that channel resources away from production per se (33). The process of risk management depends upon the scientific findings of risk assessment, as well as on public opinion. Chapter 3 describes the significant impact of public opinion, in particular local communities, on environmental applications of biotechnology. A separate OTA report surveyed public perceptions of biotechnology (48).

While recognizing that complex interrelationships between risk assessment and risk management exist, a key to improving the current process is to distinguish between those aspects of the process that are more or less scientific (risk assessment) and those that are matters of policy or value judgments (risk management). In fact, a 1983 report by the National Academy of Sciences recommended that regulatory agencies establish and maintain clear conceptual distinction between the two processes (33).

The domains of risk assessment and risk management of environmental applications of genetically engineered organisms are often blurred in debates on this controversy. **This chapter focuses on risk assessment and, more specifically, on the first step in the process of risk assessment: "risk identification." To a lesser extent, issues of risk management are considered when those issues derive from the scientific underpinnings of risk assessment.** The role of the Federal Government in managing the risks associated with the environmental release of genetically engineered organisms, an issue presently being analyzed by the General Accounting Office (15), is beyond the scope of this report. This chapter also does not assess the various domains of risk management—e.g., the economic and social benefits that could be derived from planned introductions of genetically engineered organisms.

METHODS TO ASSESS RISKS OF PLANNED INTRODUCTIONS

An orderly process for organizing and interpreting information about the potential health or environmental risks of planned introductions of genetically engineered organisms must be developed and refined. To date, the methods and models proposed for risk assessment of biotechnology have been derived principally from those developed for chemicals released into the environment (13,14, 16,17,45).

Some aspects of chemical and physical risk assessment lend themselves well to evaluating risks associated with living organisms, particularly containment and mitigation (17). Other aspects of the models do not apply well to living organisms, which reproduce and are subject to selection pressure, or die out (45). Generally the risk assessment process for planned introductions involves:

- **risk identification**—identifies the potential risk, designating its source, mechanism of action, and potential adverse consequences;
- **risk-source characterization**—characterizes the potential sources of risk, describing types, amounts, timing, and probabilities of harmful events;
- **exposure assessment**—considers exposure risks, estimating intensity, frequency, and duration of exposure to the risk agent;
- **dose-response assessment**—analyzes the relationship between amount of exposure and extent of effects; and
- **risk estimation**—estimates, within a range of uncertainty, the overall risk (14,47).

At present, standardized protocols do not exist for predicting either the kinds of risks or the magnitudes that could be associated with planned introductions of genetically engineered organisms into the environment. Chemical fate models, epidemiological models, and effects models to assess aspects of environmental applications are useful, but not complete. **Nevertheless, risk assessment is not an impossible task and conducting a flexible, case-by-case, science-based risk assessment** (19,36,40) **develops and enhances the risk assessment structure. Small-scale, experimental field testing will enhance significantly both the basic scientific database and the ability to modify existing risk assessment models.** Some argue, however, that developing an adequate assessment structure requires substantial new resources beyond those currently obligated for testing and evaluating chemical and other inert hazardous substances (16). In either case, risk assessment raises questions and illustrates data requirements that must be addressed. The implications that many of these questions have for a national research agenda are discussed at the end of this chapter.

CONSIDERATIONS TO EVALUATE IN ASSESSING POTENTIAL RISKS

Identifying and evaluating the array of elements to consider for risk assessment of environmental applications of genetically engineered organisms is one of the issues that must be addressed. Yet underlying this evaluation is the fundamental question of whether or not the entity should be regulated strictly as a product, or as a product that is special because it derives from recombinant DNA processes. Historical considerations of risk assessment and regulation indicate that process-based decisions are not logically consistent with assessments of other biological products.

But are there other important criteria that should be analyzed? Some argue that there are, and one of the first attempts to predict or classify potential environmental impacts of the introduction of genetically engineered organisms described five parameters (1):

> **Possible Negative Effects.**—Before establishing complex risk assessment schemes to address potential problems, this basic question should be examined. If there are none, then no cause for concern exists.
>
> **Survival.**—Will the engineered organisms survive when introduced? If not, then there is little likelihood that an ecological problem might arise. If, however, the organism intended for release has been designed to fit a certain role and to survive in the environment, then further assessment is required.
>
> **Reproduction.**—Many intended applications do not merely require survival or persistence, but depend on replacement or multiplication of the organism to achieve the desired endpoint. Reproduction of an organism leading to an overwhelming net increase in number could increase the probability of unintended potential effects.
>
> **Horizontal Gene Transfer** (see ch. 4).—Horizontal transfer of genetic material could be a concern even if the released engineered organisms die after performing their intended function.
>
> **Transportation or Dissemination.**—If the organisms move beyond the environment in which they were released and were intended to function, they become a potential agent for interacting with other populations or communities. This could have unintended and unpredicted consequences.

Many standards—both positive and negative—exist to gauge the value of each of these criteria, and in particular the first question. Yet, in assessing risks, uncertainties will always exist because the ability to ask questions or pose problems exceeds the ability to answer them. The risk management process must be designed to assess the value of these and other criteria and, as indicated earlier, to weigh the benefits and risks of new products against those of old ones. Nevertheless, scientific criteria important to risk assessment (especially risk identification) of planned introductions applications can be identified, and are discussed in the rest of this chapter.

WHAT RISK ASSESSMENTS AND REVIEWS ARE NECESSARY?

Basic to all arguments about the risks of deliberate release of any non-native plant or animal are risk management decisions about the kinds of hazards that are tolerable or intolerable. It is clear that some planned introductions warrant greater concern than others (32). An application with some degree of risk, but with the potential for widespread benefit, would probably be subject to detailed regulatory review. Regulatory review should also, however, be flexible. Thus, for effective and efficient regulatory review, certain applications of genetically engineered organisms could be identified as having negligible risk (e.g., see Coordinated Framework for Regulation of Biotechnology, 51 F.R. 22302). Such applications could be processed through an abbreviated review or be exempt from review.

Can categories be identified for which abbreviated review or exemption is appropriate? For example, case-by-case reviews at appropriate levels of scrutiny of planned introductions for currently accepted agricultural practices that differ only by the method used to generate a product not presently regulated might be unduly burdensome. Likewise, applications that are qualitatively identical to previously approved products, e.g., a new ice-minus bacteria application, pose no unexamined risk and should probably be subject to less review or be exempt.

A priori exclusion from review is problematic for many scientists. On the other hand, many scientists argue that applications should be assessed and classified on the basis of certain criteria, and categories requiring different levels of review be developed (23,32). This section examines risk assessment and review considerations for some types of environmental applications.

Crop Plants and Domesticated Animals

Genetically engineered crop plants and domesticated animals appear to be the most likely candidates for the category of minimal risk applications for planned introductions. Two important questions should be considered, however, before assuming that either type of release is wholly safe:

- Do the engineered crop plants or domesticated animals have relatives that occupy similar ecological niches and are problematic as weed plants or feral or pest animals?
- Are the engineered organisms toxic to nontarget species?

In addition, altering a fundamental metabolic process in plants or animals (e.g, plants engineered to fix nitrogen or mobilize a novel insoluble nutrient) could require special scrutiny. Changing metabolic processes can perturb critical energy and nutrient cycles. For example, augmenting nutrient flows might increase freshwater pollution (see ch. 5).

Finally, changes in mutualistic species of crop plants or domesticated animals should probably be carefully examined. Mutualisms—complex interactions in which both species benefit, such as pollination by insects—are important to ecosystem function (6). Introductions of genetically engineered species that interact as mutualists should be tested carefully to ensure that they are ecologically equivalent to existing partners.

Nevertheless, despite the reservations of some, introductions of genetically engineered crop plants or domestic animals are strong candidates for an abbreviated review process in the near future.

Pathogens and Pests

Pathogens or pests used for genetically engineered products slated for environmental release have the potential to affect the environment adversely. The degree of risk associated with importing *any* genes from pathogens to nonpathogens is also of concern. A distinction, however, can be made between the genes of a pathogen that are involved in the disease process and those that direct basic structural or metabolic functions. While some would argue that *any* genes used from such organisms pose problems, others point out that *general* genes of structure and metabolism are not special in pathogens.

Pathogenicity is known to involve a number of genes that must be intact and operate in a concerted and coordinated fashion in pathogens (12,39). The kind of review that relatives of pathogenic species should be subject to is a topic of much discussion. There are two kinds of relatives of pathogenic organisms: avirulent strains that differ from the pathogen by only one or a few genes, and nonpathogenic relatives that contain none of the disease-causing genes. The effect of genetic change on these two types of relatives has different potential for undesirable consequences. Nonpathogenic relatives probably have little realistic chance of acquiring all of the characteristics necessary to become pathogenic (39). That is, because pathogenicity usually requires the concerted action of several genes, a change in one gene would not be likely to convert a nonpathogen to a pathogen. Thus, nonpathogenic relatives could require less review than pathogenic organisms if all other criteria are equal.

On the other hand, genetic changes can readily convert some avirulent relatives to virulent pathogens (30,39). In these cases, the relatives are avirulent forms of the disease causing species and differ from the pathogen by only one or two changes. For example, changes in virulence have been described for viral and fungal pathogens. Temperate viruses that infect bacteria can become virulent with genetic changes at only one or a few loci (29). Certain fungal pathogens of plants can evolve (in response to selection pressure) virulence characteristics against new cultivatable varieties within a few years after the new cultivars have been widely planted (50). Finally, avirulent strains of a pathogen can recombine within an organism during an infection to produce lethal recombinants. Recent experiments demonstrated that two avirulent herpes simplex virus type 1 strains could interact in vivo to produce virulent recombinants resulting in a lethal infection in mice (21).

Changing a pathogen's environment or introducing it into a new one could potentially affect virulence expression or host range—thus arguing against exempting from review proposed releases using pathogens, pests, or avirulent relatives. Artificial and disturbed environments, in particular, seem likely settings for such shifts in expressed virulence of a pathogen. *Legionella pneumophila*, the causative agent of Legionnaire's disease, for example, occurs in natural freshwater habitats, but can also adapt to life in cooling towers and other nonnatural aquatic environments from which it has easier access to humans (43). Similarly, *Endothia parasitica* (chestnut blight) is an opportunistic fungal pathogen of several tree species. In its native Asia, the fungus causes little damage, because forest trees have evolved resistance genes (25). Since its accidental introduction into North America, the fungus has virtually eliminated the American chestnut tree throughout its geographic range in the Appalachian mountains because these trees previously had not been pressed by natural selection to evolve resistance.

Thus, the use of pathogens and some related avirulent species in environmental applications could pose special problems. Such releases are not likely candidates for exemption from review. However, **applications transferring general genes (genes not involved in causing disease) from pathogens to nonpathogens do not present pathogen-specific problems. In the absence of evidence indicating special risk factors, such applications could receive a review less rigorous than one using a pathogen.** Finally, although the release of any form of pathogenic organism as a biological control agent is likely to generate controversy, this is an area where a mathematical and genetic framework exists for examining the properties of a pathogen before release (2,3,22). Furthermore, there is extensive experience with biocontrol agents, especially in the use of soil-borne plant pathogens (42).

Molecular Construction of the Organism

A different approach that could be used to distinguish applications requiring different levels of review examines the molecular details of the altered organism's genetic construction. Such an examination could include, but not be limited to:

- whether the genetically engineered organism has been constructed using genetic material from the same genus (intrageneric) or different genera (intergeneric);
- whether the alteration of the released organism involves the insertion or deletion of genetic material; and
- whether the alteration of the released organism involves regulatory (not structural or coding) sequences.

Because applications involving genetically engineered micro-organisms have generated much of the controversy in this area of biotechnology, considerations of these criteria will focus on such applications.

Intrageneric and Intergeneric Constructions

Taxonomic groupings are in a perpetual state of flux, with the classification of organisms into species, genera, and sometimes even higher taxonomic categories often changing as scientists learn more about the phylogeny of all organisms. New molecular techniques have changed prior interpretations of evolutionary relationships—sometimes radically so. Such realignment is true for all organisms, including micro-organisms.

The degree of relatedness of the genetic material used in genetically engineered organisms has generated considerable debate. Are intrageneric organisms so similar to those that already exist in nature that laboratory-derived intrageneric constructions can be exempt from review? Or are the risks of intrageneric and intergeneric constructions similar, so that they should be reviewed in a similar manner?

Because the prospects for competition leading to extinction can be greater for closely related species than for distantly related ones (9), some propose that slightly modified organisms should receive special scrutiny. Others argue that the designation of genera in micro-organisms, and in bacteria in particular, is often arbitrary and the concern about any single case should not be on taxonomic grounds per se, but rather on the ability or inability to cause harm (12).

Intrageneric and perhaps even intraspecific genetically engineered organisms might be expected to be less affected by any fitness disadvantage arising from changes in the natural balances between the genetic components of individuals in any population. Hence, they would be more likely to persist in the environment than intergeneric recombinants. Persistence of intergeneric recombinants, on the other hand, could be less of an issue than for intrageneric recombinants (27). If persistence is an important criterion, then taxonomic factors should be considered.

Gene Deletions

The deletion of an existing gene from an organism is more likely to arise spontaneously in nature than the *de novo* acquisition of a new characteristic. **This class of molecular alterations probably poses the least risk for adverse consequences resulting from survival and reproduction after release of the engineered organisms.** Other considerations, however, such as the potential for adverse genetic, physiological, and ecological consequences of the particular application would have to be assessed before such applications could be assumed safe and entirely exempt from review. Nevertheless, some review requirements are less pertinent to this type of molecular construction than for those involving acquisition of new characteristics, and so a modified review process could be appropriate.

Regulatory Genes

Regulatory genes control when and how much structural gene product a cell makes. The levels of gene product can affect the expression of individual characteristics, as well as the overall development, structure, function, and vigor of an organism.

Because gene dosage and timing are important to an organism's development, gene alterations in regulatory sequences are important to changes in structure and function (from an evolutionary and ecological standpoint) (5,20). In one example of tetracycline resistance in the bacterium *E. coli*, changes in a regulatory gene result in a 50-fold difference in the level of resistance to the antibiotic (11). Such changes can also affect the organism's ability to survive and reproduce in competition with related or neighboring organisms (31).

Regulatory gene changes in recombinant organisms could conceivably not only produce different patterns of gene expression, but also enhance persistence in the environment. On the other hand, changes in regulatory sequences that alter the metabolic regulation of an organism can reduce the organism's fitness (and thus survivability or persistence).

A pertinent argument has been made that less concern should be focused on the regulatory gene than on the trait whose expression is controlled—some traits being beneficial, while others have the potential for harm (19). The situations just described illustrate this point. Thus, developing a strategy to review regulatory gene changes based on the trait controlled, rather than on the molecular construction of the application, might be prudent.

IDENTIFYING RISKS: MICRO-ORGANISMS V. MACRO-ORGANISMS

A staggering array of genetically engineered organisms have been proposed for potential environmental applications (see app. A; 44,46,51). Public concern over this next step in biotechnology appears greater when the release involves microorganisms. Genetically engineered plants with plant genes have been field tested with little controversy. Genetically engineered plants with bacterial or fungal genes and genetically engineered bacteria, on the other hand, have involved more controversy and have been held up longer in the regulatory system or in litigation (10). Is this perception of differential risk between micro-organisms and macro-organisms realistic? Are there actually different risks to releasing genetically engineered bacteria, algae, fungi, or viruses (micro-organisms) compared with genetically engineered plants or animals (macro-organisms)?

Ecological Impacts

The obvious distinction between micro-organisms and macro-organisms is their size, and size may affect the ecological impact of an introduced organism. For example, larger organisms may move farther, move more biomass, and cycle more nutrients (per individual) through an ecosystem. They are also relatively easier to track and recover and, in general, biologists know more about their natural history than they do about microorganisms.

Although the introduction of small organisms would probably generate less notice than the release of even a few big organisms in the wrong place, the small organisms are potentially more difficult to control. The generally more rapid reproductive rates of micro-organisms could allow

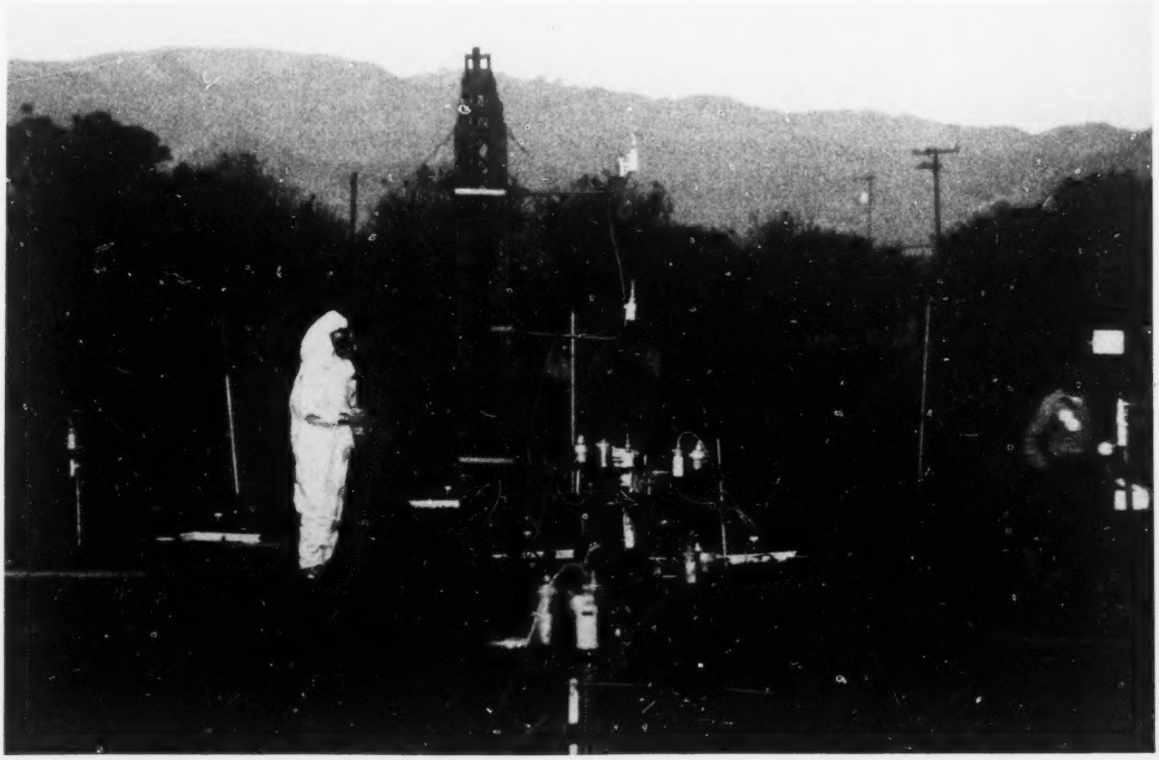

Photo credit: Peter Forde, Advanced Genetic Sciences

Some of the equipment set up for monitoring survival and dispersal of ice minus bacteria in Advanced Genetic Sciences' first field test.

them to proliferate and spread more rapidly through the environment than larger organisms. Micro-organisms are also harder to retrieve and exterminate.

Competition

Competitive interactions between newly introduced engineered organisms—either micro- or macro-organisms—and native organisms are crucial components of ecological impact assessment. Competition occurs if the released and indigenous organisms limit each other's abundance in the environment without consuming each other. Competition with alien organisms can result in extinction of native taxa (9,28,53). Among plants, competition-based local extinction appears relatively common (52). Are genetically engineered micro-organisms better able than macro-organisms to compete with natives?

Most data on this subject have focused on macro-organisms. Theories of competition do not suggest any difference between the effects of macro- and micro-organisms. Those studies that considered competition of micro-organisms also indicated that the principles of competition are universal for both types of organisms (22).

An important issue affecting the impact of released organisms on competitive interactions in the environment is that the less that is known about an ecosystem and a species, regardless of its size, the harder it is to describe competitors. This factor is not an actual function of the different sizes of micro-organisms v. macro-organisms. At present, more pertinent information for macro-organisms exists. Thus, it should be easier to assess competitive interactions (and adverse consequences) for the release of an altered macro-organism.

Cascade Effects

One of the more difficult problems in analyzing the environmental impact of intentionally introduced organisms is the possibility of cascade effects—changes in one species that destabilize relationships between other species, leading to changes in species far removed from the original disturbance. Cascade effects are the least predictable potential consequences of a planned introduction. Although ecologists have spent a lot of time examining the structure of communities, it is still rarely possible to anticipate all the ramifications of introducing or removing a seemingly innocuous species. Chapter 5 discusses cascade effects in microbial communities and among macro-organisms.

Genetic Impacts

In addition to considering different ecological impacts based on the size of the released organism, important genetic questions should be examined:

- can the organisms exchange genetic information through sexual mechanisms;
- can the organisms exchange genes between species via nonsexual mechanisms (e.g., viruses or plasmids, see ch. 4); and
- does either mechanism carry greater risk of adverse environmental consequences with macro-organisms or micro-organisms?

The issue of genetic transfer mechanisms is not one of differences between macro-organisms and micro-organisms. Instead, it is a question of problems specific to a particular release application. For example, even for applications that use sexual reproduction as the principal mechanism for genetic exchange, another factor—the mating system—must be considered. Species that require two individuals of opposite sexes to establish a breeding population do not as readily colonize distant areas as species in which a single individual can establish a breeding population.

Generalizations about potential genetic impacts, however, are best applied to well-studied species of organisms—small or large. Among wild and little-known species, there are diverse mating systems in both groups. Plants and some vertebrates are known to exchange genes with members of other genera, while bacteria in the field might do little recombining of any sort. Thus, a strategy to generically assess potential genetic risk on the basis of a distinction between macro-organisms and micro-organisms would be inadequate.

Evolutionary Impacts

The risks of evolutionary lability in the deliberate release of either micro-organisms or macro-organisms should be considered. Neither group is perfectly adapted to its environment. Given real space and finite populations, any organism is likely to evolve if presented with an environmental opportunity or challenge (7). An analysis of the environmental risks (including genetic and ecological risks) would be inadequate if the released taxa evolved characteristics that were lacking when the organisms were introduced. The potential for evolution is a function of both population size and, most importantly, the selection pressure applied. Do micro-organisms differ from macro-organisms in their potential to evolve?

Number of Organisms

The number of organisms to be introduced is a key component in the consideration of evolutionary potentials and impacts. Micro-organisms and macro-organisms include diverse types with different potential for invasion and establishment. In general, the number and the density of organisms involved in a given environmental application will be greater in a release of micro-organisms. This difference in numbers makes the probability of an evolutionary response higher in an introduction that involves micro-organisms. Other things being equal, the evolutionary risks associated with the environmental release of micro-organisms will be greater than those for released macro-organisms. For example, if mutation occurs at a rate of one in 10^7 organisms and if one in 10^3 of these mutations has an evolutionary impact, the net probability of an environmental impact is one in 10^{10}. Therefore, a release of 10^{12} bacteria in a field trial (e.g., 1 liter of 10^9 organisms per milliliter) could result in an evolutionary impact in that population. However, such a consequence is unlikely to occur among a release of only 10^9 organisms.

Selection Pressure

Selection pressure is the most critical component in estimating the probability of adverse evolutionary consequences. In the face of selection pressure, a trait that favors survival and reproduction (i.e., differential reproductive success) will increase in frequency in the population. Traits conferring disadvantages will be selected against. Natural selection appears to operate on micro-organisms and macro-organisms by the same mechanisms. But the larger number of micro-organisms (by as much as six orders of magnitude) can allow more rapid adaptive responses since they have shorter generation times and more genetic variation can be screened in a given amount of time.

Some applications are more sensitive to selection pressure than others, and this difference stems mainly from the various uses of engineered organisms. Again, however, sheer numbers of released organisms could play an important role. For example, strong selection that eliminates 99 percent of a species will leave behind 10 survivors of a population of 1,000, and 10,000 survivors of a population of 1 million. The survivors of such selection would probably be better adapted, and a population of 10,000 is more likely to reproduce itself than is one of 10. Thus, larger populations (in this case a larger number of released organisms) increase the probability of evolutionary response to selection pressure. The larger numbers of released organisms that would be associated with micro-organism applications could more readily evolve in response to environmental selection pressures than the smaller macro-organism populations.

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IMPLICATIONS FOR A RESEARCH AGENDA

Planned introductions of genetically engineered organisms are the new frontier in biotechnology. The techniques for exploiting this frontier (e.g., recombinant DNA technology and plant cell culture) are well-developed and continue to be refined. But **specific data and basic, broad-based information about many areas necessary to develop capabilities for generic risk assessment and management strategies are lacking.** In many instances, **much of this information can only be obtained by small-scale, experimental field testing.** Still, controversies surrounding initial applications to release genetically engineered organisms into the environment have illuminated areas where research efforts should focus.

Current Status

Although nearly all risk assessments—including chemical assessments—are and will continue to be plagued by incomplete data (8), some research efforts have begun to yield information that is critical to the examination of potential risks associated with the planned introduction of genetically engineered organisms. As the data compiled from small-scale field trials increase, regulators can develop and refine the risk management process. The charge of funding research efforts to enhance risk assessment of environmental applications of genetically engineered organisms falls principally to three Federal agencies: the Environmental Protection Agency (EPA); the U.S. Department of Agriculture (USDA); and the National Science Foundation (NSF).

Environmental Protection Agency

EPA funds biotechnology risk assessment research through the Office of Research and Development. **At EPA, all biotechnology research projects—intramural and extramural—involve risk assessment.** Funding in fiscal year 1986 totaled $4.4 million; in fiscal year 1987 it reached $5.7 million (26). Table 6-1 lists some of the types of projects being funded by EPA to develop an adequate scientific database that will allow prediction of environmental risks possibly associated with the deliberate release of genetically engineered organisms. In a recent solicitation for research grant proposals, EPA identified ecological risk assessment, ecosystem structure and function, and ecological and toxicological effects as priority areas (49).

Table 6.1.—Types of U.S. Environmental Protection Agency Biotechnology Risk Assessment Projects

- Methods for assessing the fate of genetically engineered micro-organisms in the soil
- Methods for detecting, identifying, and enumerating genetically engineered bacteria in the soil
- Survival, modification, and effects of genetically engineered micro-organisms in aquatic environments
- Genetic transfer in aquatic environments
- Fate and effects of genetically engineered micro-organisms on ecological processes
- Fate and effects of genetically engineered micro-organisms in simulated natural environments

SOURCE: Office of Technology Assessment, 1988.

Federal law limits the scope of EPA's biotechnology research efforts to those program projects designed to assist other EPA sectors to perform their missions. Nevertheless, the range of research projects that the Agency funds should provide valuable data and should advance in the field of biotechnology risk assessment and risk management generally.

U.S. Department of Agriculture

Biotechnology research performed by or for USDA is administered chiefly through the Agricultural Research Service and the Cooperative State Research Service. Direct risk assessment components of research represent only a small fraction of the biotechnology projects at either agency (4,47). No clear direction to increase or promote risk assessment aspects of biotechnology research at USDA exists (4). Recent reports, however, indicate that the Department plans to increase the profile and funding in its research agenda of aspects specific to biotechnology risk assessment (18).

National Science Foundation

From 1 to 13 percent of biotechnology projects funded by NSF relate directly to risk assessment (47). Equally as important as funding for direct biotechnology risk assessment, however, is NSF funding for research projects to develop the fundamental scientific database needed for appropriate risk strategies. NSF supports projects in several key areas, including microbial ecology, the ecology of genes and plasmids, evolutionary biology, and systematics. Additionally, NSF has issued a program solicitation to establish Biological Facilities Centers and Biological Research Centers (35), one of which is to be devoted to biotechnology risk assessment (24).

Finally, NSF, USDA, and the U.S. Department of Energy have undertaken a joint initiative for plant science centers beginning in fiscal year 1988 (34). Successful initiatives might involve examining responsible application of genetic engineering to avoid undesirable environmental effects or clarifying ecological processes in agroecosystems and (34).

Research Needs For the Future

Several permits for small-scale field tests of environmental applications of biotechnology are in the regulatory approval process or have been been approved and conducted (see ch. 3 and app. A). While the information and data gained from these experiments will be valuable in designing future risk assessment and risk management protocols, an expansion of basic scientific research to gather data critical to risk assessment is also necessary. Billions of dollars are being invested throughout U.S. industrial sectors to comply with various environmental standards, but comparatively little is being invested specifically to improve the scientific basis for the standards (8). An improvement in risk assessment would be a major step in ensuring that compliance money is well spent (8). Important areas of research include:

- **Taxonomy and Systematics.**—gathering data on the classification and evolutionary relationships of natural populations (especially nontarget micro-organisms) should be emphasized.
- **Natural History.**—collecting data on the life histories of organisms intended for planned introductions and the organisms with which they interact is critical, especially for those with potentially harmful impacts.
- **Ecology.**—the complex interactions of microbes, plants, and animals need to be better understood so that improved predictive capabilities can be developed. Crop ecology, in particular, is important because agricultural uses will be the majority of early applications.
- **Test Systems.**—aquatic and terrestrial laboratory microcosms and mesocosms look promising for analyzing ecological processes prior to planned introduction. They need further development and refinement.

However, **although increased investment in specific research areas is critical, the paramount need is to develop interdisciplinary research programs in order to support biotechnology risk assessment.** Research in such programs should be thoroughly integrated, from hypothesis generation to experimentation and interpretation of results. Active research cooperation among microbiologists, geneticists, ecologists, molecular biologists, evolutionary biologists, plant pathologists, entomologists, agronomists, and epidemiologists should be encouraged. The scientific data gained through such collaborations would improve significantly the ability to assess biotechnology risks (37,38,41). Nevertheless, uncertainties and questions in risk assessment of planned introductions will continue to exist. No amount of research funding can answer all questions; tough risk management decisions that weigh benefits against risks will need to be made.

SUMMARY AND CONCLUSIONS

During the 1970s, public concern about the effects of technology on the environment and human health led to the development of methods to help regulators evaluate and control potential hazards. More recently, attention has focused on evaluating risks that might be associated with planned introductions of genetically engineered organisms into the environment. The related issue of managing risk has also received public attention.

Risk assessment, a process distinct from risk management, uses scientific data to estimate the effects of exposure to hazardous materials or other situations. Derived from a factual base, it can be qualitative or quantitative. Risk management, on the other hand, weighs policy alternatives to select the most appropriate regulatory strategy or action. This process depends on the scientific findings of risk assessment, as well as the role of the public. It involves the integration of scientific, social, economic, technical, and political concerns. A key to improving the current situation is to distinguish between those aspects of the process that are scientific (risk assessment) and those that are matters of policy decisions (risk management).

Although risk assessment of recombinant DNA technology is not a new issue,.the specific application of risk assessment to planned introductions of genetically engineered organisms has become a matter of debate. Methods developed in the previous decade for chemical risk assessment have been suggested as models for assessment of risks that might result from the planned introduction of engineered organisms. To date, however, **generic risk assessment protocols to analyze the impacts of genetically engineered organisms have not been widely agreed upon.**

Experiences with historical agriculture applications are pertinent and provide parallel scientific information for many potential applications. Small-scale, experimental field testing is necessary to improve baseline data for risk assessment.

At present, some scientists argue that the expectation of safety can best be met by a scientific review of proposed releases of genetically engineered organisms into the environment on an adaptable, case-by-case basis, and that at this time *a priori* exclusion of any application from review is problematic. Other scientists hold that a rigid case-by-case approach could paralyze advances in planned introductions of genetically engineered organisms, and that a proper balance between regulation and safety would involve each organism being assessed and classified (on the basis of scientific criteria) into categories requiring different levels of review. In either case, future experience with the impact of various types of genetically modified organisms on environmental processes should result in a safe, streamlined review process for some applications, or a lifting of the requirement for review.

Categories that could be considered for abbreviated review include: crop plants and domesticated animals, and organisms involving nondisease-associated genes or gene deletions. Applications in which the genetically engineered product is substantially identical in its properties to naturally occurring genetic variants; that could be produced with previously existing methods and without being subject to regulation under existing law; that are already available and approved for field testing; or that contain no new genetic material except marker sequences in noncoding regions probably could warrant abbreviated review or possibly even exemption.

Ecological, genetic, and evolutionary impacts that could result from planned introductions of micro-organisms or macro-organisms are important in assessing risks of an introduction. In general, existing knowledge of macro-organisms (plants or animals) exceeds information available for micro-organisms (bacteria, algae, fungi, or viruses), so less scrutiny might be required to yield an evaluation of the risks associated with the release of a macro-organism.

Controversies surrounding the initial applications to release genetically engineered organisms into the environment have illuminated areas where fundamental knowledge of many systems is currently lacking. In particular, active research cooperation among microbiologists, geneticists, agricultural scientists, plant pathologists, entomologists, agronomists, ecologists, and evolutionary biologists should be encouraged. **Interdisciplinary research is critical to developing adequate risk assessment and risk management for planned introductions of genetically engineered organisms.** To dispel speculation, increasing the general knowledge base about organisms intended for environmental applications is paramount.

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Appendixes

Appendix A

Pending and Potential Planned Introductions of Genetically Engineered Organisms

The information contained in this appendix is grouped according to the organism receiving the transferred genetic material, or, in the case of deletions, the organism from which genetic material is deleted. Within any given entry, pending applications (those at or near the field test stage) are described first. In some cases, material on likely or possible applications follows. This information was compiled from publicly available materials, and was last updated in early 1988.

Microbes

Bacteria

Killed Bacteria as Pesticide (Mycogen, San Diego, CA).—Mycogen scientists cloned the delta-endotoxin gene from *Bacillus thuringiensis* (BT) and inserted it into a strain of *Pseudomonas* via plasmid-mediated transformation. The *Pseudomonas* is cultured to produce large amounts of the delta-endotoxin, a protein that is toxic to lepidoptera larvae. The bacteria are then killed, their cell walls fixed, and the resulting 'poison capsule' is administered as a topical insecticide. Using the killed and fixed bacteria as a delivery vehicle increases the longevity of the toxin in the environment. In other applications the protein is rapidly degraded by sunlight and other environmental action. Using killed bacteria also eliminates most containment problems. Small-scale field trials were conducted in test plots near Orlando, FL.

Toa Gosei Chemical, in Japan, has an active research program aimed at producing similar killed BT agents. They are screening natural strains of *B. thuringiensis* to identify varieties that will target specific pests (e.g., diamond back moth, fabricius, etc.) and cloning the toxin genes into *Escherichia coli* and *B. subtilis* to enhance production (37).

"Ice Minus" (Advanced Genetic Sciences (AGS), Oakland, CA).—AGS researchers have produced strains of *Pseudomonas syringae* and *P. fluorescens* from which the gene for the ice-nucleation protein has been deleted. AGS had hoped to field test in early 1986, but legal challenges, local opposition, and controversy over unauthorized facilities for pathogenicity tests mandated by the Environmental Protection Agency (EPA) caused delay. The first field test began on April 24, 1987, near Brentwood, in Contra Costa County, CA.

The bacteria were topically applied to 2,400 strawberry plants to test the degree of frost resistance conferred upon strawberry plants in the 0.2-acre plot, as well as to monitor such risk assessment parameters as survival and dispersal. The manipulations involved do not impart any new genetic information; rather, they delete existing information. Comparable strains of bacteria occur naturally, the results of random mutation. Potential for ultimate survival or spread of test strains has been judged low. There is no likelihood of novel characters being transmitted to nontarget species. EPA announced approval of the application for an experimental use permit in February 1987.

A similar experiment was first proposed in 1982 by Steven Lindow and Nickolas Panopoulos of the University of California at Berkeley to delete the gene for the ice-nucleation protein from strains of *P. syringae* and *Erwinia herbicola* and field test them for increased frost resistance of host plant substrates (especially potato plants). EPA and the Recombinant DNA Advisory Committee of the National Institutes of Health both approved the proposal. Local opposition and legal challenges by the Foundation on Economic Trends made a Fall 1986 test impossible. The field test commenced on 29 April 1987, near Tulelake, CA, with the planting in a half-acre plot of 2,000 inoculated tubers and 2,000 controls.

"Ice plus" (Snomax Technologies, Oakland, CA).—Naturally occurring strains of spray-dried *P. syringae* (produced by Kodak Bioproducts Division for AGS), containing high concentrations of the ice-nucleation protein, are mixed with water and sprayed from snowmaking guns at ski resorts. This procedure is from 20 to 80 percent more efficient than water alone, and has been tested on ski slopes in Colorado, Michigan, Minnesota, New York, and Vermont. The 1986-87 season saw large-scale use in a number of resorts (a major delay until recently has been in production facility availability and capacity). Use has been approved on U.S. Forest Service lands. It is also possible that the bacterium might be used in a variety of ice-making applications in the Arctic.

AGS claims they have also developed a recombinant-DNA form of the bacterium that is up to 1,000 times as effective as the wild-type strain. There are no immediate plans to use this in any environmental applications.

Engineered Microbial Pesticide (Monsanto, St. Louis, MO).—Monsanto scientists have cloned the delta-endotoxin gene from *B. thuringiensis* and used transposable elements to install it in the chromosomes of strains of *P. fluorescens*, a microbe that colonizes the surfaces of corn plant roots. In its host, the inserted gene is expressed and the gene product retains its toxicity. Monsanto plans to inoculate seed corn with the engineered bacteria in the hope that they will colonize the roots of the developing plant. The insecticidal activity of the protein toxin should then protect the plant from the corn-root cutworm. Monsanto's proposed field test would involve planting 27,000 coated seeds on a 1-acre test plot for each of two consecutive years.

While data on the behavior of transposable elements in other systems (notably *Drosophila*) suggest caution is warranted, potential problems associated with horizontal gene transmission seem to have been preempted in this case. Monsanto scientists inserted the delta-endotoxin gene into the *Pseudomonas* chromosome with a disarmed transposable element—one from which the transposase gene (necessary for element mobility) was deleted. Insertion was affected with the aid of a "helper" transposable element, from which one of the long terminal repeats necessary for insertion was deleted (25,26,27).

Monsanto has submitted to EPA a proposal for an Experimental Use Permit for field testing. EPA has asked for data from additional toxicity protocols, which are now being performed.

Toxic Waste Disposal.—Bacteria may be engineered to enhance or receive capabilities to metabolize or sequester specific toxic wastes, including polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxin, oil spills, phenol, pesticides, herbicides, and heavy metals. A host of bacteria capable of metabolizing different substrates are known (17). One such bacterium is a strain of *Pseudomonas* (developed by Ananda Chakrabarty) that has been given the capability (plasmid-mediated) to metabolize several components of crude oil. This may enhance the possibilities to control oil spills biologically. It differs from established techniques by combining several different carbon degradative pathways into one organism. Existing methods generally use a mixture of naturally occurring bacteria each with the capacity to metabolize a different crude-oil component. EPA researchers in Gulf Breeze, FL, have developed systems capable of degrading jet fuel, 2,4,5-T (the active ingredient in Agent Orange), trichloroethylene (a solvent that is the most common ground water contaminant), and pentachlorophenol (PCP, a common wood-treatment chemical).

Many naturally occurring bacteria have the ability to degrade a variety of chemicals found in toxic waste. These abilities could be enhanced both by classical selection and genetic engineering. Species of *Achromobacter* are able to degrade carbofuran, a pesticide against corn rootworm and other crop pests. *Flavobacterium* species are known to degrade coumaphos, a pesticide used against livestock pests. An atrazine resistance gene has been isolated from cyanobacteria, or blue-green algae. Such bacteria may be engineered for environmental cleanup programs, such as degrading toxic material remaining in storage tanks where farmers dump pesticide or herbicide wastes.

The EPA lab at Gulf Breeze has developed a "suicide" plasmid to ensure that a gene encoding a 3-chlorobenzoic acid-degrading enzyme will be destroyed when it has served its purpose. The plasmid contains four elements: the gene for the degradative enzyme, a promoter, a methylase gene, and a restriction enzyme-encoding gene. In the presence of 3-chlorobenzoic acid, the promoter induces production of both the degradative enzyme and the methylase, which protects the degradative gene from the action of the restriction enzyme. When the acid has been completely degraded, methylase production is suppressed, and the restriction enzyme digests the degradative gene. Scientists believe this type of scheme should be applicable to many other metabolic pathways, though there are no imminent plans to field test such microbes.

Toxic Waste Disposal or Heavy Metal Recovery (Mining).—Bacteria might be engineered to enhance their abilities to extract or concentrate heavy metal contaminants from land fills or mine tailings. The bacteria would promote the efficient recovery of minerals, minimize existing pollution, and leave reclaimed land usable for agriculture. A number of existing mining operations use bacteria to aid in the recovery process, such as cobalt extraction with *Thiobacillus ferrooxidans*. Other potential targets include lead, cadmium, and copper.

Researchers at the EPA laboratory in Gulf Breeze, FL, are developing bacterial strains resistant to mercury, with the intention of using them to reduce pollution problems in mercury-contaminated environments.

It may also be possible to alter some species of algae to enhance or impart toxic waste disposal ability (see section on Plants: Engineered Algae).

Pollution Control.—Phosphorus removal, ammonia oxidation, and flocculation are three significant problems facing municipal water purification systems. Bacteria could be engineered to aid each of these tasks.

Miscellaneous.—Ecogen (Princeton, NJ) is using both classical and biotechnological approaches to develop a variety of novel pesticides. "Plasmid curing" has produced some new BT toxin vehicles, and recombinant DNA techniques are being used to investigate potential pesticide uses of *B. subtilis* toxin genes.

After normal drilling operations, as much as 50 percent of the oil may remain in wells, due to capillary forces and chemical adhesion (8). Engineered bacteria may help extract that remaining oil.

Microbes may be tapped to aid in biomass energy production, as with yeast engineered to enhance their ability (generally in contained facilities) to produce ethanol for fuel. Scientists at the University of Florida and elsewhere are currently studying this possibility.

Bacteria may also be engineered to enhance their function in food production processes (28).

Viruses

Viruses as Pesticides.—By manipulating the organization and expression of baculovirus genomes, researchers hope to increase baculovirus utility as pesticides with specific applications. Commenting on potential risks, one researcher has said "My guess is no problem but there are a variety of constructs possible, and some could have broader effects than desired. . . . One can consider many scenarios. In the worst case (also the most improbable) the situation could not be corrected. . . . In the course of our studies already we have developed what we consider improved methods of assessing risks of genetically engineering viral pesticide products. . . . Our methods of assessing viral gene expression in nontarget hosts are extremely sensitive" (19). Work is being done in this area by at least three academic groups (in Florida, Texas, and Georgia) and two commercial companies (Genetics Institute, Cambridge, MA, and MicroGeneSys, West Haven, CT).

Researchers at the Institute of Virology in Oxford, England, are pursuing several similar applications. The first involves a baculovirus that is pathogenic to the pine beauty moth, *Panolis flammea*, a pest of lodgepole pine (and other species), especially in northern Scotland. Field tested viruses contain special marker nucleotide sequences, to aid in tracking distribution and dispersal. Although approval has been obtained from the appropriate regulatory bodies (the Advisory Committee on Genetic Manipulation of the Health and Safety Executive, and the Forestry Commission) as well as the Nature Conservancy Council, delays made testing impossible in 1986. Field tests took place during 1987. (British regulation is largely voluntary, and somewhat more flexible than at present in the United States.)

A similar test to measure viral persistance in a given ecosystem involved a baculovirus (*Autographa californica* nuclear polyhedrosis virus) pathogenic to caterpillars of the mottled willow moth, *Spodoptera exigua*. This test was carried out by Institute of Virology scientists at an undisclosed United Kingdom site during the summer of 1986 (3). In both cases, the scientists anticipate inserting genes to increase the virulence of the viral pathogen. The gene for the BT delta-endotoxin is a likely candidate.

Genetics Institute (Cambridge, MA) is pursuing research aimed at inserting an insect toxin gene into a nuclear polyhedrosis virus. The engineered virus is targeted at *A. californica*, an alfalfa pest, as well as *Heliothis* species, pests of various crop plants including cotton, soybeans, and rice. Similar work on related viruses is under way at Tottori University, in Japan.

Commonwealth Scientific and Industrial Research Organization (CSIRO) researchers in Australia are exploring genetic engineering techniques as a means of restoring the virulence of the myxoma virus. Originally introduced to control pest populations of introduced rabbits, attenuated forms of the initially virulent virus evolved at the same time rabbits developed increased resistance. Although the original virus devasted rabbit populations, the subsequent coevolution between host and pathogen has led rabbit numbers to once again increase. Scientists are attempting to add a bacterial toxin to the viral genome to increase its potency (42).

Viruses in Engineered Vaccines.—On 23 July 1986, the Food and Drug Administration (FDA) announced approval of "Recombivax HB®," a genetically engineered vaccine for hepatitis B, a disease carried by as many as 200 million people worldwide, 700,000 in the United States alone. Based on the hepatitis B cell surface antigen, the vaccine was developed by Chiron (Emeryville, CA) and marketed by Merck & Co. It has been shown to produce the same high level of immunity in clinical trials as Merck's blood-plasma-based vaccine, but without the potential worries associated with contamination of donated blood by the AIDS (acquired immunodeficiency syndrome) virus. Several Japanese groups are working on an engineered hepatitis B vaccine, as are researchers at the Pasteur Institute, Paris; at Merck, Sharp, & Dohme in West Germany; and at Smith Kline Biologicals, Belgium (29).

A cooperative group at the Japan Polio Research Institute and the University of Tokyo Medical School has recently announced the construction of an attenuated polio virus vaccine that retains high immunogenicity. Additional competitors are emerging (40). Japanese research is also focussed on engineered herpes simplex viruses as vaccines against several human diseases.

Chiron is also working on the development of vaccines against hepatitis A, AIDS, malaria, and oral and genital herpes. In October 1986, Chiron announced a joint venture (the Biocine Company) with Ciba-Geigy to produce genetically engineered vaccines.

Researchers at the National Institute of Allergy and Infectious Disease (NIAID) and elsewhere have inserted into the vaccinia virus (used originally for smallpox vaccinations) genes responsible for antigen production in a number of diseases—herpes simplex, influenza, hepatitis B, rabies, and respiratory syncitial virus, all in humans; and vesicular stomatitis in cattle, horses, and pigs. Vaccinia virus is particularly valuable as a vector because it needs no refrigeration; it is cheap, easy to administer, has a large capacity for foreign DNA; and it has proved safe and effective in over 200 years of use. An early case of inadvertent release involved the accidental vaccination of laboratory researchers during a test in mice of a vaccine for vesicular stomatitis (13).

A vaccine for malaria is being pursued by several research programs, including groups at New York University, Chiron, NIAID (working with vaccinia systems), and a collaborative effort by Smith, Kline & French and the Walter Reed Army Institute of Research. Preliminary human trials began on 17 March 1986, with a vaccine developed by the latter. Early reports of results were disappointing.

Peter J. Hotez and colleagues at Rockefeller University are developing a vaccine against hookworm, two species of which (*Necator americanus* and *Ancyclostoma duodenale*) cause a substantial public health problem worldwide. The antigenic determinant for the vaccine is the histolytic proteolytic enzyme (HP), which attaches the hookworm to the wall of the small intestine. Synthetic antigen in the vaccine stimulates the host to produce antibodies to the enzyme. Researchers used phage lambda gt11 to clone the gene for HP necessary for large-scale production.

Australian scientists at CSIRO, working with Biotechnology Australia and Arthur Webster, two Australian biotechnology companies, have developed a vaccine for sheep foot rot, caused by *Bacteroides nodosus*. The gene from this bacterium, encoding the production of the cell surface filaments crucial to infection, was transferred to *P. aeruginosa*. Inoculating sheep with the altered *P. aeruginosa* bacterium stimulates the desired immune response.

Different groups in the European Economic Community are pursuing engineered vaccines for the chicken pathogen, infectious bronchitis virus (IBV), foot and mouth disease, bovine and swine rotavirus, bovine leukemia, avian erythroblastosis, swine pseudorabies, and rabies.

Scientists at Baylor University and at Texas A&M field tested a vaccine for swine pseudorabies in 1984. An investigation by the National Institutes of Health's (NIH) Recombinant DNA Advisory Committee found the researchers remiss for not having consulted the local Institutional Biosafety Committees, and concluded that ambiguities in the NIH guidelines contributed to confusion in the case. The vaccine was developed by Novagene, Inc. (Houston) and is now commercially available through TechAmerica Group, Inc. (Omaha, NB).

In 1986, researchers from Oregon State University, at the invitation of collaborators in New Zealand, field tested an engineered vaccinia virus in that country. The research was designed to study immunogenicity, pathogenicity, and transmissibility of a model vaccinia virus in sheep, calves, and chickens. The engineered vaccine contained structural genes for proteins from sindbis virus, a single-stranded RNA virus (31). The researchers found the tests successful.

Scientists from the Wistar Institute (Philadelphia) developed, and collaborators from the Pan American Health Organization field tested, an engineered vaccinia virus vaccine against rabies in Argentina in 1986 (see ch. 3).

Vaccines (especially those using vaccinia virus) may be engineered to carry simultaneously the antigenic determinants for a series of diseases, related or not (20,30). These vaccines must be monitored carefully, to avoid the possibility of recombination producing newly virulent viral forms (12).

In the face of antigenically complex systems, such as malaria or sleeping sickness, engineered multivalent vaccines may be the only way to provide general protection. Work is under way to produce such engineered vaccines (41), but estimates place successful completion 10 or more years in the future. However, preliminary successes with previously refractory diseases, such as schistosomiasis (27), may mean that single antigen approaches might be fruitful against systems once thought amenable only to multivalent vaccines.

Plants

Herbicide Resistance

Glyphosate.—Scientists at Calgene (Davis, CA) have inserted the *aro*A gene from *Salmonella typhimurium* into tobacco to confer tolerance to the herbicide glyphosate (e.g., Monsanto's Roundup®). A disarmed plasmid from *Agrobacterium rhizogenes* served as the vector (7). While this gene transfer imparts a new characteristic to strains of an existing plant species, the chance that these characteristics might spread seem to be very low, if not zero. The U.S. Department of Agriculture (USDA) approved Calgene's applications to field test the altered tobacco plants.

Calgene has patented the *aro*A gene under the name GlyphoTol® and plans to file applications soon to test similarly transformed tomato and cotton plants. Corn, soybean, rape, and some trees are targets of similar efforts. Calgene has also been working on transforming oil rape (*Brassica napus*) with a bacterial antibiotic resistance gene.

Researchers at Monsanto have used the Ti plasmid from *A. tumefaciens* to insert genes for glyphosate resistance into tobacco, tomato, and petunia cells from which resistant plants have been regenerated (34). Field tests were conducted in the summer of 1987.

Atrazine (Ciba-Geigy).—The herbicide atrazine, manufactured primarily by Ciba-Geigy and marketed as AAtrex, is degraded very slowly in the environment. An effective herbicide, annual sales of AAtrex amount to nearly $250 million, making it the second best selling herbicide after Roundup®. Atrazine is commonly used on corn, which is naturally resistant. But because it persists in soil, it can contaminate runoff and reduce yields in sensitive crops that are planted in rotation with atrazine-treated corn, a problem termed "carry-over." Several crops are likely candidates for the induction of atrazine resistance. According to one estimate, atrazine resistance in the prominent varieties of soybeans would allow sales of the herbicide to double or triple (21). Field tests of engineered atrazine resistance in tobacco were carried out by Ciba-Geigy in North Carolina in 1986.

The gene for atrazine resistance was transferred by cross-breeding from bird's rape (*Brassica campestris*) to oil rape, or canola (*B. napus*), which was field tested on 30,000 acres in Canada in 1984.

Sulfonylurea (Du Pont).—Genes for resistance or tolerance to the sulfonylureas, such as chlorsulfuron (Glean®, used on grains, especially wheat and barley) and Oust© (a broad spectrum herbicide), are the targets of research aimed at producing transgenic plants. Soil residues of these herbicides can damage crops grown in rotation, such as sunflowers or soybeans. Engineering resistance into rotation crops would protect them and expand the market for these herbicides. Work on this possibility has so far involved the production of plants transformed with resistance genes introduced into tobacco cells produced in tissue culture. Du Pont, collaborating with Northrup King, field tested resistant tobacco in 1987.

Miscellaneous.—Researchers at Molecular Genetics (Minnetonka, MN) are pursuing the induction of resistance to imidazolinone (made by American Cyanamid) in corn. Approaches based on cell culture and selective breeding are closest to commercialization.

Calgene (Davis, CA) scientists have isolated a gene conferring tolerance to bromoxynil, a herbicide made by Rhone-Poulenc Agrochimie, France. Several strategies are being explored to introduce the gene into sunflower plant cells and other plant tissues. USDA has approved field tests scheduled for 1988 of tobacco plants engineered to be tolerant of this herbicide.

In January 1987, scientists at Plant Genetic Systems, Belgium, reported the genetic engineering of tomatoes, potatoes, and tobacco plants "totally resistant" to the broad spectrum herbicide "Basta®," produced by Hoechst AG, Frankfurt am Main, West Germany. The active ingredient in the herbicide, phosphinotricin, inhibits the plant enzyme glutamine synthetase. Phosphinotricin can be inactivated by acetylation. The gene for an acetylating enzyme has been isolated from a strain of streptomyces and inserted into target plants, protecting them from doses of Basta® as high as 10 times those used normally for weed killing (23).

Disease Resistance

Crown Gall Resistant Tobacco (Agracetus, Middleton, WI).—Agracetus scientists used a disarmed Ti plasmid to transform tobacco with a gene conferring resistance to crown gall disease. NIH approved the company's application for small-scale field testing in 1986, and USDA's Animal and Plant Health Inspection Service (APHIS) reviewed the same proposal and certified it as involving "no plant pest risk." Field testing began on 30 May 1986, in Middletown, WI. Agracetus is pursuing similar ends in corn and soybeans.

Agracetus scientists have also transformed cotton plants (Gossypium hirsutum) with an antibiotic resistance gene coding for neomycin phosphotransferase (38).

Engineered Viral Cross Protection (Monsanto/Washington University St. Louis, MO).—Researchers at Washington University, in collaboration with Monsanto, have engineered cross protection against TMV in tobacco plants. Resistance to TMV infection was conferred by inserting the TMV coat protein gene into the tobacco plant genome via the Ti plasmid from *A. tumefaciens* (1). Similar results have been achieved with tomato mosaic virus and alfalfa mosaic virus, suggesting that many plants can be engineered to produce viral disease resistance. A field test of tomatoes engineered to be resistant to tobacco mosaic virus was approved by USDA and begun on a one-third-acre plot near Jerseville, IL, on 2 June 1987.

Miscellaneous (Rothamstead Experimental Station, Harpenden, United Kingdom).—Researchers have used cell fusion to produce a potato variety resistant to the potato leaf roll virus. The parental strains are a domestic potato and a wild, South American potato. Britain's Advisory Committee on Genetic Manipulation approved a field test for the summer of 1987. Also approved were field tests to be carried out at the Plant Breeding Institute (Cambridge) of potatoes carrying two added bacterial enzymes, as models for possible future improvement efforts.

Pest Resistance

BT Toxin Protected Crops.—In 1985, the Belgian company Plant Genetic Systems acheived the first introduction of the delta-endotoxin gene from *B. thuringiensis* into a plant.

Rohm & Haas, collaborating with Plant Genetic Systems, has since used the Ti plasmid to insert the delta-endotoxin gene into tobacco plants, providing protection primarily against the tobacco hornworm, *Manduca sexta*. Field testing was successfully completed in 1986 in Dade County, FL, and Bolivar County, MS. Hybrid seed is expected to reach the market in the 1990s.

Monsanto researchers have recently used the Ti plasmid from *A. tumefaciens* to insert the BT toxin gene into tomato plants, to provide protection against lepidopterous pests. Field tests were carried out in the summer of 1987 (11).

Miscellaneous.—Plant geneticists in England (a collaboration between Agricultural Genetics Company and the Plant Breeding Institute, both in Cambridge) have used *A. tumefaciens* vectors to insert into tobacco plants a gene coding for a protein that is a natural inhibitor of insect trypsin, a digestive enzyme. The spectrum of insect resistance thus conferred is much broader than that of BT-toxin-based applications (24). Seeds might be engineered to produce or increase their natural production of other "antifeedants," such as canavanine, thus reducing losses of stored seeds or grain to insect pests.

Other researchers at the Plant Breeding Institute have begun 1987-88 field tests of potatoes into which a kanamycin resistance gene has been inserted, with an *A. tumefaciens*-derived vector, to aid in risk assessment and agronomic studies of transgenic forms of a well established cultivar.

Tolerance to Environmental Factors

Plants can be engineered to increase their tolerances to such limiting environmental factors as salinity, drought, or sensitivity to heavy metal toxicity. This artificial expansion of ecological niches could be exploited to bring marginal lands into agricultural use or to decrease problems of deforestation and erosion due to overexploitation (in the Sonora, Great Basin, Negev, and Sahel, for example).

Nitrogen Fixation Enhancements

The promise of using genetic engineering techniques to enhance the nitrogen fixation in some plants, and to bestow it on others, has been highly publicized. Symbiotic bacteria in nodules on the roots of nitrogen-fixing plants extract gaseous nitrogen from the atmosphere and convert it to chemical forms accessible to plants. The biochemical pathways in bacteria that perform this function usually involve 16 or 17 structural genes and their associated regulatory sequences, usually referred to as the *nif* (nitrogen fixing) genes.

BioTechnica International (Cambridge, MA) has produced two potentially commercial strains of *Rhizobium meliloti*, a bacterium that forms nodular colonies on the roots of alfalfa plants. The strains have been engineered to enhance their nitrogen-fixing ability through the insertion, via disarmed plasmid vectors, of additional copies of their own regulatory genes. The company hopes that 1988 field tests will demonstrate a 15- to 20-percent increase in nitrogen-fixing ability.

British scientists at the Rothamstead Experimental Station have inserted a marker sequence into a strain of *Rhizobium* for a summer 1987 field test to monitor the extent of gene transfer between rhizobial strains in soil.

Much of the research aimed at imparting nitrogen-fixing ability to plants that do not have it naturally is focused on transferring the *nif* genes into the plant. Formidable technical problems are involved in transferrring so much genetic material and ensuring its proper expression. Substantial progress with this more generally applicable approach may be 5 to 10 years away.

Engineered Algae

Donald Cheney and colleagues (Northeastern University, Boston, MA) are using protoplast fusion techniques to tailor marine algae (especially *Dunaliella salina*) to increase the efficiency of production of beta-carotene, agar, and other algal byproducts, and George Melville (Australian National University, Canberra, and Westfarmers Algal Biotechnology Pty., Ltd., Perth) are using recombinant DNA techniques to achieve the same goal.

Michael T. Henzl and Benjamin Greene, at New Mexico State University, have described the ability of the common alga, *Chlorella vulgaris*, to sequester gold. Similar abilities to sequester other heavy metals are known in other alga (36). Genetic manipulation may one day be able to enhance such abilities.

Miscellaneous

Researchers at Michigan Technological University are exploring the use of *Agrobacterium* vectors to impart new qualities to larch trees. The objective is to induce disease and herbicide resistance, making this rapid growing, genetically malleable conifer more valuable for reforestation programs.

Fungi are being explored, both by classical methods (David Sands, Montana Sate University) and cell fusion techniques (Gary Harman, Cornell) as herbicides targeted against Canada thistle and spotted knapweed and as antidisease agents, respectively. In the latter case, cell fusion methods were used to produce hybrid strains of the soil fungus *Trichoderma harzianum* that will be applied via inoculation on the seeds of peas and cucumbers. Field tests at the New York State Agricultural Experiment Station Vegetable Research Farm near Geneva, NY, will test the ability of the inoculated strains to protect against diseases such as damping off and root rot, caused by other fungi. The Environmental Protection Agency approved the field tests on 8 September 1986.

Japanese scientists have used cell fusion techniques to produce a hybrid between red and Chinese cabbage, called "Bio-Hakuran." The new plant displays many characteristics that are intermediate, but it contains a full chromosomal complement from each parent—18 and 20, respectively, for a total of 38. Researchers are developing the hybrid cabbage as a new truck crop.

Genetic engineering may be able to improve the nutritional value of some plants by increasing their content of seed storage proteins and other components—for example, lysine in corn. Engineering may increase forage crop efficiency by enhancing digestibility. Plants might also be engineered to function as producers of pharmaceuticals or specialty chemicals, such as particular oils or storage lipids (14). Gene engineering of oil-seed crops for quality and quantity of oils is being done by USDA, Sungene, Calgene, Unilever, and BioTechnica, Canada.

Animals

Fish

Heat-Shocked Salmon.—Supported partly by the American Tackle Manufacturers Association, fisheries researchers in Washington and Michigan are using heat shock to induce triploidy in developing salmon embryos (Coho (*Oncorhynchus kisutch*), and king, or Chinook (*Oncorhynchus tshawytscha*)). The chromosomal abnormalities induced by heat shock disrupt normal reproductive cycles, including the spawning runs that lead to death. Plans are to stock Lake Michigan with triploid fingerlings in the expectation that more trophy fish will result. Triploid fish, with their disrupted reproductive cycles, contain no new genetic material, and cannot produce offspring. One researcher has suggested that future stocking programs should use such triploids to eliminate reproductive competition with and potentially negative impacts on the gene pools of wild salmon (16).

Triploid grass carp, also produced by heat shock, are being studied for use as aquatic weed control agents in southern riverways and irrigation systems, especially in California and Florida (39).

Miscellaneous.—Other work on salmon aims at enhancing growth hormone production, either by introducing foreign structural genes or enhancing the function of existing regulatory genes. Work with striped bass and trout is attempting to increase their cold tolerance by inserting a gene derived from winter flounder. One researcher has stated that within 5 years it should be possible "to routinely introduce genetic traits into cultured and wild fish species" (35). A number of different laboratories, most outside the United States, are pursuing work of this sort (15).

Livestock

The technology exists to genetically alter farm animals to improve reproductive performance, weight gain, disease resistance, or coat characteristics (USDA, Beltsville; University of Washington; University of Pennsylvania; CSIRO, Australia)(5). Fertilized embryos from rabbits, sheep, goats, cattle, and pigs have already been successfully transformed with human growth hormone genes (10,18). Scientists in Australia are working on moving sheep growth hormone genes between different varieties of sheep (9).

A significant body of research is being directed towards engineering livestock animals to function as new pharmaceutical sources (6), although aspects of the isolation and purification of such products remain to be worked out (4).

Poultry

Researchers in the Poultry Research Laboratory, in East Lansing, MI, have succeeded in transforming developing chick embryos with the avian leukosis virus and with chick syncitial reticuloendotheliosis virus, both common disease-causing organisms in poultry (32,33). The demonstration of the retroviral vector derived from avian leukosis virus may make it possible to inoculate chickens against the virus, as well as to insert other genes of interest, such as those regulating growth or egg production rates or conferring resistance to other diseases.

Miscellaneous

Genetic engineering techniques hold promise for altering insect pests to serve as tools in pest control or eradication. Sterile male blowflies can be produced by mutagenesis and selection. These altered flies, expected to help eradicate the pests, were field tested over 1985-86 on Flinders Island, Australia, by a group of Australian scientists (22). Efforts to control tephritid pests with engineered medflies are under way at the University of Hawaii.

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Appendix B

List of Contractor Documents

For this report, OTA commissioned eight papers on various topics concerning the planned introduction of genetically engineered organisms. One was published as *New Developments in Biotechnology, 2—Background Paper: Public Perceptions of Biotechnology*. The manuscripts of the remaining seven contract documents are available in two volumes from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA, 22161 (703) 487-4650.

Vol. 1

- Andow, D.A., Snapp, S.S., and Teng, P.S., 1986, Potential Environmental Impacts of Widespread Releases of Non Ice Nucleating Bacteria in Agriculture.
- Upper, C.D., Hirano, S.S., and Vali, G., 1986, An Assessment of the Impact of Large-Scale Applications of Ice-Minus Bacteria and Other Procedures Designed to Decrease Population Sizes of Ice Nucleation Active Bacteria on Crops.

Vol. 2

- Norse, E., 1986, Ecological Issues Relevant to Environmental Application of Genetically Engineered Organisms. Including:
  —Sharples, F.E., Relevance of Lessons From Introductions of Exotic Species.
  —Pimm, S.L., and Levin, B.R., Impact on Competitive Abilities of Specific Genetic Alterations.
  —Keeler, K., and Dobson, A.P., The Big and Small of It: Are There Different Risks to Releasing Genetically Engineered Macro-organisms and Micro-organisms?
  —Lenski, R.E., and Istock, C.A., Are There Specific Genetic Modifications That Are Inherently So Safe As To Need No Review?
- Coleman, D.C., and Hodson, R.E., 1987, An Ecosystems Approach to Potential Perturbations of Energy Flow and Nutrient Cycles Associated With Environmental Applications of Genetically Engineered Organisms.
- Gosz, J.R., Dahm, C.N., and Flanagan, P.W., 1987, Ecological Impact of Genetically Engineered Organisms on Ecosystems.
- Massey, A., and Gould, F., 1987, The Genetic Basis of Changes in Host Range or Habitat.

Appendix C

Acknowledgments

OTA would like to thank the members of the advisory panel and working group who commented on drafts of this report, the contractors who provided material for this assessment, and the many individuals and organizations that supplied information for the study. In addition, OTA acknowledges the following individuals for their comments on drafts of this report:

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Appendix D

Glossary of Acronyms and Terms

Glossary of Acronyms

AGS —Advanced Genetic Sciences, Inc.
APHIS—Animal and Plant Health Inspection Service, U.S. Department of Agriculture
BSCC —Biological Sciences Coordinating Committee
BT —*Bacillus thuringiensis*
CSIRO—Commonwealth Scientific Industrial Research Organization, Australia
DHHS —U.S. Department of Health and Human Services
DNA —Deoxyribonucleic acid
DOE —Department of Energy
EPA —Environmental Protection Agency
FDA —Food and Drug Administration
FIFRA —Federal Insecticide, Fungicide, and Rodenticide Act
FSIS —Food Safety Inspection Service, U.S. Department of Agriculture
HP —Histolytic proteolytic enzyme
IBV —Infectious bronchitis virus
INA —Ice nucleation active
NAS —National Academy of Sciences
NEPA —National Environmental Policy Act
NIAID—National Institute of Allergies and Infectious Diseases
nif —Nitrogen fixation
NIH —National Institutes of Health
NSF —National Science Foundation
OSHA —Occupational Safety and Health Administration
OSTP —Office of Science and Technology Policy
OTA —Office of Technology Assessment
PAHO—Pan American Health Organization
PBB —Poly-brominated biphenyls
PCB —Poly-chlorinated biphenyls
PL —Public Law
PMN —Premanufacture Notice
RAC —Recombinant DNA Advisory Committee, National Institutes of Health
R&D —Research and development
RNA —Ribonucleic acid
S&E —Science and Education Division, U.S. Department of Agriculture
TMV —Tobacco mosaic virus
TSCA —Toxic Substances Control Act
UK —United Kingdom
U.S.C. —United States Code
USDA —United States Department of Agriculture
UV —Ultra-violet

Glossary of Terms

Aerobic: Growth or activity that requires the presence of free oxygen.

Algorithm: A step-by-step procedure for solving a problem or modeling a process.

Allele: One of several possible alternate forms of a particular gene (e.g., blue and brown are alleles of the gene for eye color).

Anaerobic: Growth or activity that does not require free oxygen.

Anticodon: A triplet of nucleotides on transfer RNA molecules that binds to the complementary codon on messenger RNA during the polypeptide-producing (translation) process. The amino acid carried by the transfer RNA is inserted into the growing polypeptide chain. See *codon*.

Bacteriophage: A virus whose host is a bacterial cell; also called phage.

Baculovirus: A virus of the family Bacloviridae found only in invertebrates, and presently being pursued as a potential biocontrol agent. (See app. A.)

Chloroplast(s): Those structures within plant cells where photosynthesis occurs; contain small circular DNA molecules that replicate independently.

Chromosome(s): The physical structure(s) within a cell's nucleus, composed of DNA-protein complex, and containing the hereditary material—i.e., genes; in bacteria, the DNA molecule in a single, closed circle (no associated protein) comprising a cell's genome.

Codon: A series of three adjacent nucleotides in a DNA molecule that directs ("codes for") the insertion of a specific amino acid into a growing protein chain.

Conjugation: The reproductive process by which DNA is transferred between bacteria during cell-to-cell contact.

Conjugation tube: The bridge-like structure by which cell-to-cell contact is maintained during conjugation.

Conjugative plasmid: A plasmid capable of initiating and directing the process of conjugation. (Compare *nonconjugative plasmid*.)

Convergent evolution: The evolution of similar structures or similar life strategies in unrelated species because of adaptation in response to similar selection pressures (e.g., in similar ecological habitats).

Cryptic plasmid: A plasmid of undetermined function.

Cultivar: Often used to refer to plant strains. See *strain*.

Cytoplasm(ic): The substance within a cell external to the nuclear membrane; pertaining to or contained in the cytoplasm.

Disarmed transposon: A transposon altered in a manner rendering it incapable of movement, most often by deletion of the transposase gene, which encodes the enzyme necessary for excision. (Compare *transposon*.)

DNA (deoxyribonucleic acid): The molecule that is the repository of genetic information in all organisms (with the exception of a small number of viruses in which the hereditary material is ribonucleic acid—RNA). The information coded by DNA determines the structure and function of an organism.

Dominant: An allele or characteristic whose expression prevails over alternatives for a given trait. (Compare *recessive*.)

Endogenous: Developing or originating within the organism, or arising from causes within the organism.

Endotoxin: Poison produced by some gram-negative bacteria, present in the cellular membrane, and released only upon cell rupture; composed of complex lipopolysaccharide (fat-like molecule + sugar molecule) and more heat-stable than protein exotoxins. (Compare *exotoxin*.)

Episome: A DNA molecule that may exist either as an integrated part of a chromosomal DNA molecule of the host or as an independently replicating DNA molecule (plasmid) free of the host chromosome.

Epistasis: The interaction of genes at different loci resulting in the masking of a character.

Epizootic: A disease affecting many animals of one kind at the same time; analogous to the term epidemic in human populations.

Eukaryote: An organism with membrane-bound, structurally discrete nuclei and well-developed organelles. Eukaryotes include all organisms except viruses, bacteria, and blue-green algae. (Compare *prokaryote*.)

Exotic: Describing a species not originating in the place where it is found; a nonnative, introduced species.

Exotoxin: A poison excreted by some gram-negative or gram-positive organisms; composed of protein. (Compare *endotoxin*.)

Extrachromosomal DNA: DNA not associated with the chromosome(s) (e.g., plasmid DNA or organelle (mitochondria or chloroplast) DNA).

Feral: Pertaining to organisms in the wild.

F factor: See *fertility factor*.

Fertility factor: An episome capable of transferring a copy of itself from its host bacterial cell (an F+ cell) to a bacterial cell not harboring an F factor (an F− cell). When the F factor is integrated into the host chromosome (the resulting cell is called an Hfr cell), the factor is capable of mobilizing transfer of the bacterial chromosome to an F− cell.

Gamete: A mature reproductive cell (haploid set of chromosomes) capable of fusing with a similar cell of opposite sex to yield a zygote; also called a sex cell.

Gene: The fundamental unit of heredity; an ordered sequence of nucleotide base pairs to which a specific product or function can be assigned.

Gene family: A group of related genes exhibiting a high degree of homology in function and nucleotide base sequence.

Gene pool: The sum total of genes in a breeding population.

Gene probe: A molecule of known structure and/or function used to locate and identify a specific region or nucleotide sequence of a genome; usually a piece of complementary DNA that has been labeled with a tracer substance, such as a dye or radioactive label.

Genetic code: The manner in which DNA or RNA represents, through chemical subunits, information that is translated into protein. The genetic code is read in groups of three nucleotides called codons, each of which specifies a single amino acid. See also *codon, nucleotide*.

Genetic variance: The fraction of the phenotypic variance due to differences in the genetic constitution of individuals in a population. (Compare *phenotypic variance*.)

Genome: The genetic content encoded in a haploid organism's genes; in eukaryotes, a haploid set of chromosomes.

Genotype: The sum total of genetic information contained in an organism; the genetic constitution of an organism with respect to one or a few loci under consideration. (Compare *phenotype*.)

Germ line: The earliest, primitive stage of development; pertaining to tissues or cell lineages producing gametes. (Compare *somatic*.)

Gram-negative/positive: A classification of bacteria based on differential staining utilizing the Gram-Wiegert procedure. Primarily as a result of an organism's cell membrane structure, gram-negative organisms stain red and gram-positive organisms stain purple.

High copy number plasmid: A plasmid present in multiple copies within a single host bacterium. Copy number (single, low, and high) is dependent on both plasmid and host cell factors.

Homologous sequence: Nucleic acid segments having an identical or nearly identical linear order of nucleotide base pairs.

Homology: Degree of relatedness in appearance, function, or structure.

Horizontal transfer: The passage of genetic material from one organism to another via nonsexual mechanisms.

Host: In the context of recombinant DNA technology, the organism used for growth and reproduction of virus, plasmid, or other foreign DNA source.

Hybridization: A procedure in which single-stranded nucleic acid segments are allowed to bind to homologous sequences, forming hybrid double-stranded helices; also, a process (e.g., in plants, producing offspring resulting from a cross between two genetically unlike entities); the formation of new cells resulting from fusion of whole cells or cell parts of different genotypes.

Ice-minus (ice−): A bacterium lacking a functional gene coding for a protein that promotes the formation of ice crystals by providing a physical nucleus around which ice crystallizes. The gene has been deleted from strains of *Pseudomonas syringae Pseudomonas fluorescens*, and *Erwinia herbicola*, the organisms around which debate recently has focused.

Ice-plus (ice+): A bacterium with an intact, functional ice-nucleating gene.

Introgression: The entry or introduction of a gene or genes from one population into another (most often in nature via sexual reproduction, or hybridization).

IS (insertion sequence): One of a class of different nucleotide sequences found in bacteria that are capable of spontaneous movement from one chromosomal location to another. Chromosomal material may be mobilized during IS movement; movement may result in mutation at the original and/or new site(s) of insertion. (Compare *transposable element*.)

Line: See *strain* and *cultivar*.

Locus (pl. loci): The physical location on a chromosome occupied by a particular gene or its alleles.

LTR (long terminal repeat): One of a class of nucleotide sequences (300-1200 base pairs in length) associated with tumor viruses and cellular oncogenes that promote gene activity and are similar to transposons. (Compare *transposable element*.)

Meiosis: The process by which chromosomes are duplicated then divided during the formation of haploid cells (gametes).

Mendelian: Referring to a trait that is controlled by a single gene, and that shows a simple dominant/recessive pattern of inheritance.

Mendelian genetics: Classical method of observing inheritance of a trait(s) in the offspring of crosses between individuals differing in that trait(s); results in accordance with Mendel's laws.

Mitochondrion/dria: Those structures within eukaryotic cells where energy is produced and stored; contains small circular DNA molecules that replicate independently.

Natural selection: The process of differential reproductive success by which genes in a population increase or decrease in frequency with the passage of generations, depending on their contribution to the survival of offspring in which they are carried; arguably the most important of the several mechanisms by which evolution takes place, discovered by Darwin and first described in 1858-59.

Nonconjugative plasmid: A plasmid incapable of initiating or directing the process of conjugation. (Compare *conjugative plasmid*.)

Nontransferable plasmid: See *nonconjugative plasmid*.

Nucleic acid: A macromolecule composed of sequences of nucleotide bases; DNA or RNA.

Nucleotide (base): The unit of nucleic acids. The molecules consist of one of four bases—adenine, guanine, cytosine, or thymine/uracil (DNA/RNA) attached to a phosphate-sugar group. The sugar group is deoxyribose in DNA; in RNA it is ribose.

Nucleus: The membrane-enclosed structure in eukaryotes that contains the chromosomes.

Organelle: A structure in the cytoplasm of a cell that is specialized in its ultrastructure and biochemical composition to serve a particular function (e.g., mitochondria, endoplasmic reticulum, chloroplast).

P element: A transposable DNA sequence present in the fruit fly, *Drosophila*. (Compare *transposon*.)

Pathogenic: Able to cause disease; often utilized to express inactivation or lethality.

Phage: See *bacteriophage*.

Phenotype: The observable characteristics of an organism produced by the interaction of the genotype and the environment. (Compare *genotype*.)

Phenotypic variance: The variation among genetically identical individual organisms in external appearance caused by the interaction of environment with the genotype during development. (Compare *genetic variance*.)

Phylogenetic: Referrring to genetic similarities between different organisms as a result of descent from a common ancestor in evolutionary history.

Plasmid: An extrachromosomal, circular piece of DNA found in the cytoplasm and capable of replicating and segregating independently of the host chromosome. (Compare *conjugative; cryptic; high copy number; nonconjugative; nontransferrable plasmid*.)

Pleiotropy: The production of diverse phenotypic effects produced by a mutation in a single gene.

Polymerase: An enzyme that assembles a number of similar or identical subunits into a macromolecule (e.g., DNA polymerase and RNA polymerase).

Primary producer: An organism (plant or micro-organism) that uses photosynthesis to convert solar energy into chemical energy that can be used by nonphotosynthetic organisms (e.g., humans).

Prokaryote: An organism lacking organelles and whose DNA is not enclosed within membrane-bound, structurally discrete nuclei. Bacteria, viruses, and blue-green algae are prokaryotes. (Compare *eukaryote*.)

Recessive: An allele or characteristic whose expression occurs only in the absence of a dominant trait. (Compare *dominant*.)

Recombinant DNA: Hybrid DNA sequences assembled in vitro from different sources; or hybrid DNA sequences from the same source assembled in vitro in a novel configuration.

Recombination: The formation of a new association of genetic material; usually applied to the process of meiosis, during a stage of which the genetic material packaged into gametes is mixed and reconstituted in any of an enormous number of possible combinations.

Replicon: A genetic element possessing sequences specifying the initiation and control of the process by which DNA is precisely duplicated.

Retrovirus: A family of viruses whose genetic material is RNA and is further characterized by the presence of reverse transcriptase in the virion; also called tumor virus.

Reverse transcriptase: An enzyme capable of directing the production of a single-strand DNA copy from an RNA template.

Rhizosphere: A region of the soil closely surrounding plant roots that is affected by root excretions.

RNA (ribonucleic acid): A molecule existing in three forms—messenger RNA, transfer RNA, and ribosomal RNA—responsible for translating the genetic information encoded in DNA into a protein product; the hereditary material of some viruses.

Selective advantage: An organism's increased probability of reproduction and producing offspring, conferred by its genetic characteristics.

Selective pressure: The influence of factors extrinsic to an organism (i.e., environmental factors) on its ability to compete with other organisms for reproductive success.

Sibling species: Independent, reproductive populations that are genetically distinct from one another yet very closely related, and often difficult or impossible to distinguish by morphological or other criteria.

Somatic: Pertaining to all diploid cells of an organism except the germ line. (Compare *germ line*.)

Species: Taxonomic category subordinate to a genus composed of individuals with common characteristics that distinguish them from other groups of the same taxonomic level; in sexually reproducing organisms, a group of interbreeding natural populations that are genetically distinct from other such groups.

Strain: A pure culture of organisms within a species, characterized by one or more particular physical or genetic properties. See *cultivar*.

Toxin: See *endotoxin* and *exotoxin*.

Transduction: The transfer of genetic material from one cell to another by means of a virus or bacteriophage.

Transformation: Introduction and assimilation of DNA from one organism into another via uptake of naked DNA.

Transposable element: A class of DNA sequences capable of insertion into a genome at numerous positions, and of moving from one area of a genome to another area or another genome.

Transposase: Enzyme that assists movement of DNA during the process of transposition.

Transposon: A type of transposable element incapable of autonomous existence, often shuttling genetic material back and forth between cell chromosomes, between smaller replicons, and between chromosomes and replicons.

Triplet: Three consecutive bases along a nucleic acid chain. See *codon, anticodon*.

Vector: A DNA molecule used to introduce foreign DNA into host cells.

Vertical transfer: The passage of genetic material from one organism to another through the germ line, i.e., sexual mechanisms; in bacteria, through genome replication and cell division.

Virus: Any of a large group of organisms containing genetic material but unable to reproduce outside a host cell.

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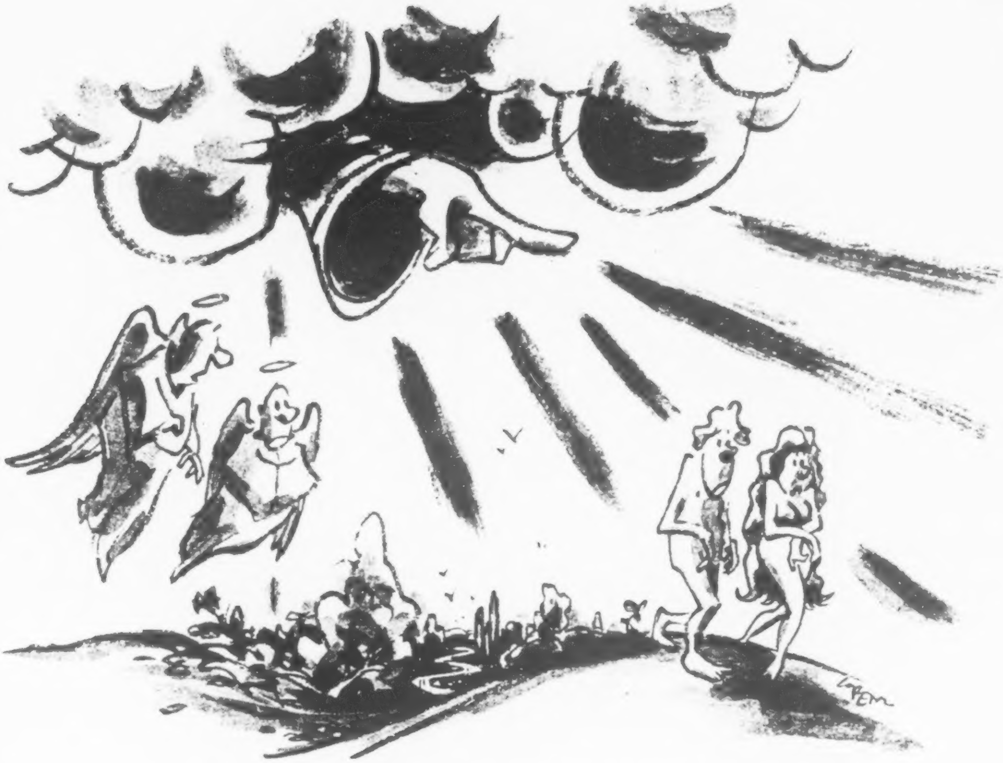

"Frankly, I think we'll regret introducing these organisms into the environment."

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